ADAPTATION OF INVASIVE SPECIES TO ISLANDS AND THE PUERTO RICAN HONEY BEE

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EDITORIAL
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Editorial: Adaptation of Invasive Species to Islands and the Puerto Rican Honey Bee

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Keywords: biological invasions, Caribbean, Africanized honey bee, *Apis mellifera*, pollinators

Editorial on the Research Topic

Adaptation of Invasive Species to Islands and the Puerto Rican Honey Bee

Honey bees critically impact global food security as pollinators in agricultural systems worldwide (Aizen and Harder, 2009; Potts et al., 2016). They are also considered one of the most successful invasive organisms, having been transported by humans to all continents except for the Arctic and the Antarctic. However, along with other insects, they are increasingly under threat by anthropogenic activities (Wagner et al., 2021). We explored the adaptation of honey bees introduced by humans to the Americas.

Africanized honey bees (AHB) are the product of human introduction of *Apis mellifera scutellata* bees from Africa to the Americas in 1956. These African bees later hybridized with European honey bees previously brought to the Americas and resulted in the hybrid AHB, infamous for their high defensive behavior and serious economic and ecological impact (reviewed in Guzman-Novoa et al.). The adaptation of AHB to the island of Puerto Rico, such as the reduced defensive behavior, provides insight into changes that can occur to invasive organisms and the invaded ecosystem after colonization (Rivera-Marchand et al., 2012; Avalos et al., 2017). An advantage of research focused on island populations is that adaptive processes on islands are accelerated and may readily show similar patterns across species. Examples of adaptations on islands include the breakdown of the usually observed mutualism between *Cecropia* trees and *Azteca* ants and the reduced aggression of *Solenopsis geminata* on Puerto Rico (Rivera-Marchand et al., 2012 and references therein). Thus, data from island populations can be particularly useful to develop and test models of invasion biology.

This collection of research articles was inspired by the "Puerto Rico Honey Bees and Evolution of Invasive Organisms on Islands" conference, held in July 2019, in person, in Puerto Rico (PRHB, 2019). The focus of the conference was the Puerto Rico Gentle Africanized honey bee and other non-native organisms, through the lens of invasion biology and island biogeography. This Frontiers Research Topic broadens the scope of the conference presentations by including new organisms, data, and perspectives, in the post-pandemic world. Several articles were completed and submitted before the pandemic, and some produced under pandemic conditions, resulting in a collection of papers with publication dates in 2020 and 2021.

This Frontiers Research Topic highlights the study of island invasion biology from the perspective of different disciplines and approaches, including genomics, morphology, behavior, ecology, and long-term data analyses. The result of this interdisciplinary approach is an

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In Memoriam:
This paper is dedicated to the memory of Prof. Modesto Matias (1951–2022).

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examination of: 1. The invasive species on the island of Puerto Rico; 2. Ancestral populations of the invasives; 3. Adaptations of invasives and characteristics of Puerto Rico honey bees.

THE INVASIVE SPECIES ON THE ISLAND OF PUERTO RICO

Three articles examine invasive species in Puerto Rico. Zimmerman et al. review invasive species of Puerto Rico in general, based on long-term research in El Yunque National Forest. Next Ackerman reviews the positive and negative impacts of honey bees on other bees and plants in Puerto Rico and the Caribbean. The negative and positive effects of invasives in Puerto Rico are explored by Cabrera-Asencio and Meléndez-Ackerman using mango cultivars and their honey bee pollinator.

Zimmerman et al. using data from the El Yunque National Forest, conclude that the invasives they examined were able to establish in Puerto Rico independent of disturbance of habitat. An exception to their findings were vascular plants, determined to be less likely to invade minimally disturbed forest habitat. The presence of honey bees as well as other invertebrates, was not influenced by level of disturbance.

The impact of the successful invasive honey bee on islands is reviewed by Ackerman. This review, with its presentation of positive and negative effects, highlights the need for future research on the impact of invasive bees on islands. Honey bees, when present, add to the resilience of pollination networks, as in the case of a key rainforest resource, the Sierra Palm (*Prestoea montana*). However, the effectiveness of honey bees as pollinators may also have negative results by facilitating the establishment of undesirable invasive plants.

The influence of honey bee pollination on invasive plants can be illustrated using the introduced mango cultivar in Puerto Rico. Honey bee pollination was not considered important to the productivity of this agricultural commodity. However, the work of Cabrera-Asencio and Meléndez-Ackerman, demonstrated a 90% decrease in honey bee visits to mango flowers after Hurricane María, a finding concomitant to a 60–70% decrease in fruit production.

ANCESTRAL POPULATIONS OF INVASIVES

The ancestral populations that gave rise to the Puerto Rico honey bee include the highly mixed European and Africanized honey bees from the Americas (Acevedo-Gonzalez et al., 2019). Guzman-Novoa et al. outlines the process and outcomes of the Africanization in Mexico while Bianchi et al. demonstrate the potential variation in a continental population that include phenotypes and genotypes like those found in the Puerto Rico population. Furthermore, this admixture variation can be compared with patterns exhibited by locally adapted populations of honey bees in their native range, as discussed by Kükrer et al. for honey bees in Turkey.

POTENTIAL “PRE-ADAPTATIONS”

Puerto Rico has two prominent social insect invaders, fire ants and honey bees (Torres and Gaud, 1998). Ortiz-Alvarado and Rivera-Marchand, discuss the unique behavioral plasticity of fire ant queens in Puerto Rico. In this species, in Puerto Rico, unlike in any other described ant species, queens demonstrate worker behaviors in response to colony demographic changes. The flexibility of this characteristic can function as a pre-adaptation that may contribute to a successful invasion.

Honey bee, physiological, morphological, and behavioral traits may contribute to their success across the world and in Puerto Rico. Smith et al. explore the morphological characteristics of honey bee mandibles that enable them to bite and inflict damage to their parasitic mites *Varroa destructor*. The biting behavior and associated resistance of Africanized (Guzman-Novoa et al.) and Puerto Rico honey bees (Rivera-Marchand et al., 2012) to *V. destructor* are now well-established characteristics. In this issue, Russo et al. also discuss this topic with reference to honey bees from Argentina. The morphological basis that underpins the *V. destructor* resistance has only recently begun to be explored.

Temperature and seasonal effects on physiology and behavior are usually thought of as important for honey bees in temperate zones. However, these aspects may become relevant under the novel contexts honey bees encounter in island environments. Saleem et al. explore the role of temperature in altering the toxicity of commonly used neonicotinoid insecticides. Feliciano-Cardona et al. explore the importance of the seasonal production of long-lived bees for colonies with respect to seasonal resource availability, even in the absence of a temperate winter.

An important feature of invasion biology is the ability of invaders to find and mate with conspecifics in the new environment. The work of Galindo-Cardona et al. addresses the risks and benefits of sexual reproduction for honey bees, with respect to finding mates and transmission of disease. Galindo-Cardona et al. show that in their study sites in Argentina, findings obtained from drone congregation areas reflect the health status of colonies present in the same study areas.

This Frontiers Research Topic is a testament to the rich research base that honey bees provide and can lead to interdisciplinary and integrated examinations of invasion biology of this one species. The knowledge regarding the impact and adaptations of introduced populations may also answer practical concerns such as the movement of bees for apiculture and agriculture. In fact, the conference helped to catalyze the formation of a technical working group to study risks and benefits of honey bee movement in the example of one country, i.e. the United States. (Marcelino et al.). The example of honey bee invasive biology can also lead to significant and novel research and applications for other biological invasions on islands.

AUTHOR CONTRIBUTIONS

RG, AG-C, EM-A, S-CC, and TG contributed to the conception and design of the special issue. All authors contributed to the editorial writing and revision, read and approved the submitted version.

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REFERENCES

Acevedo-Gonzalez, J. P., Galindo-Cardona, A., Avalos, A., Whitfield, C. W., Rodriguez, D. M., Uribe-Rubio, J. L., et al. (2019). Colonization history and population differentiation of the Honey Bees (Apis mellifera L.) in Puerto Rico. *Ecol. Evolut.* 9, 10895–10902. doi: 10.1002/ece3.5330

Aizen, M. A., and Harder, L. D. (2009). The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. *Curr. Biol.* 19, 915–918. doi: 10.1016/j.cub.2009.03.071

Avalos, A., Pan, H., Li, C., Acevedo-Gonzalez, J. P., Rendon, G., Fields, C. J., et al. (2017). A soft selective sweep during rapid evolution of gentle behavior in an Africanized honeybee. *Nat. Commun.* 8, 1550. doi: 10.1038/s41467-017-01800-0

Potts, S. G., Imperatriz-Fonseca, V., Ngo, H. T., Aizen, M. A., Biesmeijer, J. C., Breeze, T. D., et al. (2016). Safeguarding pollinators and their values to human well-being. *Nature*. 540, 220–229. doi: 10.1038/nature20588

PRHB. (2019). *Puerto Rico Honey Bee and Invasive Species on Islands Conference*. Available online at: https://prhb.cs.fiu.edu/

Rivera-Marchand, B., Oskay, D., and Giray, T. (2012). Gentle Africanized bees on an oceanic island. *Evolut. App*. 5, 746–756. doi: 10.1111/j.1752-4571.2012.00252.x/full

Torres J. A., and Gaud, S. M. (1998). Los insectos de Puerto Rico. *Acta Científica* 12, 33–41.

Wagner, D. L., Grames, E. M., Forister, M. L., Berenbaum, M. R., and Stopak, D. (2021). Insect decline in the Anthropocene: Death by a thousand cuts. *PNAS*. 118, e2023989118. doi: 10.1073/pnas.2023989118

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Worker Queens? Behavioral Flexibility of Queens in the Little Fire Ant *Wasmannia auropunctata*

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Many species of social Hymenoptera demonstrate behavioral flexibility, where older workers that typically forage can revert to younger worker tasks, such as nursing, when these are absent. This flexibility is typical of the sterile worker class, yet rare in queens. In the little fire ant (*Wasmannia auropunctata*), queens have been reported to perform only egg laying. We examined behavior of queens of *W. auropunctata* after demographic manipulation. When half of the workers were removed from the colony, queens were observed caring for eggs, larvae and pupae as well as eating outside of the nest, like forager workers. We examined the relationship between these atypical queen behaviors and their juvenile hormone binding protein (JHbp) and vitellogenin (Vg) expression via QRT-PCR method. *JHbp* and *Vg* expression decreased when queens were performing worker tasks, resembling the expected expression pattern of typical sterile workers. Flexibility in queen behaviors in the little fire ant may be an important adaptation to changing environments. As a significant invasive species, such adaptation may increase the probability of colony survival during propagation. Our results not only present new insights in behavioral flexibility in social insects, but also increases our understanding of the success of this significant invasive species.

Keywords: behavior, flexibility, invasive, ant, queen, juvenile hormone, vitellogenin

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INTRODUCTION

Eusocial insects are characterized by having reproductive division of labor (Wilson, 1971). Within the colony one or more individuals carry out egg laying while sterile workers perform nest related tasks including queen and brood care (i.e., nursing), defense and foraging (Gordon, 1996). Division of labor in workers may be associated with age or morphological differences (Hölldobler and Wilson, 1990). Nevertheless, there are species where workers show behavioral flexibility, performing tasks that are not typical of their age or morphology. For example, in honey bees (*Apis mellifera*), nurses are known to forage precociously when foragers are absent, while forgers may revert to nursing according to the needs of the colony (Robinson, 1992). Similar behaviors has been observed in the eusocial wasp *Polybia occidentalis* (O'Donnell, 2001). Workers of the ant *Pheidole dentata* are found to increase their behavioral repertory as they age, where older workers perform tasks typical to younger individuals, yet the young workers are not proficient in older worker tasks (Calabi and Traniello, 1989; Seid and Traniello, 2006; Mertl and Traniello, 2009). Although behavioral flexibility in workers is an important adaptation that increases the chances of colony survival when the worker population decreases, it has not been reported in queens (Rüppell et al., 2002).

Queens produce eggs throughout most of their life. In species where the colony goes through a founding stage or independent colony foundation, queens perform worker type tasks until workers are reared. For example, in the red imported fire ant, *Solenopsis invicta*, the founding queen starts with nest construction, lays and tends the first batch of eggs until these emerge as adult workers, which take over worker duties. From this point on queens are known to only lay eggs (Tschinkel, 2006). Contrastingly, in species that reproduce through colony budding or dependent colony foundation, where one or more queens depart from the main colony with a group of workers, the queens do not experience a founding stage (Keller, 1991; Peeters and Ito, 2001). Since these queens keep a group of workers at all times it is not expected that they perform worker-like tasks.

We examined queen behavior in *Wasmannia auropunctata*, the little fire ant, a native of South America and an aggressive invasive species on all other continents except Antarctica (Le Breton et al., 2003; Wetterer and Porter, 2003; Mikheyev et al., 2008). Its colonies are composed of 200–500 monomorphic workers and one to twelve larger queens, and reproduce by colony fission (Wetterer and Porter, 2003; Foucaud et al., 2006; Mikheyev et al., 2009). Older workers, which typically carry out foraging duties, demonstrate behavioral flexibility by performing nursing duties when young workers (i.e., nurses) are absent (Rivera-Marchand and Fernández-Casas unpublished). The first objective of this study was to determine if queens of *W. auropunctata* demonstrate behavioral flexibility, performing worker tasks when necessary. Since colonies of the little fire ant do not experience a solitary founding stage (Wetterer and Porter, 2003; Foucaud et al., 2006; Mikheyev et al., 2009), queens typically have no need to perform worker tasks. We did not expect queens to perform worker duties until we had observed queens manipulating eggs. Based on these preliminary observations we expected that in the absence of workers, queens of *W. auropunctata* should have the behavioral flexibility to perform worker tasks.

Reproductive division of labor in eusocial Hymenoptera (ants, bees, and wasps), is under endocrine control (e.g., JH and Vg; Bloch et al., 2002; Amsalem et al., 2014). Juvenile hormone (JH) and vitellogenin (Vg) have important roles in regulating insect physiology (Dolezal et al., 2009, 2012) such as development, reproduction, and behavior (Robinson and Vargo, 1997; Dong et al., 2009; Azevedo et al., 2016). JH, considered a master hormone, has been found to control behavioral development in honey bees (Robinson and Vargo, 1997; Sullivan et al., 2000). It influences physiology in queens and guarding behavior in workers of primitive eusocial wasp *Polistes canadensis* (Giray et al., 2005). JH also affects queen maturation and reproduction in the invasive ant *S. invicta* where, high levels of JH induces alates to begin oogenesis (Vargo and Laurel, 1994; Brent and Vargo, 2003; Lu et al., 2009). Vg is a yolk precursor protein; its production is typically used to produce egg yolk by oviparous animals (Amdam et al., 2003), but it may also affect behavior (Nelson et al., 2007). In honey bees Vg has a role in the reproductive division of labor where concentrations are correlated with the hierarchy of the hive and reproductive division of labor (Corona et al., 2007; Nelson et al., 2007). Similar trends of Vg expression are seen in ants of *S. invicta* (Lewis et al., 2001; Lu et al., 2009) and *Pogonomyrmex* spp. (Corona et al., 2013; Libbrecht et al., 2013). Therefore, the second objective of this study was to measure gene expression of JH and Vg in relation to the tasks performed by queens. We expected egg laying queens to have higher levels of JH and Vg than worker-like queens.

MATERIALS AND METHODS

Samples

Nests ($N = 20$) of the little fire ant *W. auropunctata* were collected from dry twigs and leaf litter in the northern region of the Caribbean island of Puerto Rico. They were housed in artificial nests which consisted of plastic boxes (25cm × 13cm × 7.5cm) coated with Fluon (Northern Products Inc., Alsip, IL, United States) on the sides. The boxes' lids were perforated with a pin to allow air exchange. The nesting area within the nest box consisted of a 3 cm^2 piece of thin (less than 1 cm thick) wood elevated 0.5 cm by a strip of clay placed along the edges of the wood. Each nest was kept at 25°C, a relative humidity (RH) between 80 and 85%, and 12 h light cycles. Nests were fed daily with 0.5 g of feeding mixture containing agar, eggs, honey, and vitamin supplements (Hölldobler and Wilson, 1994) placed in a feeding arena within the box at approximately 10 cm from the nesting area. Nests used in the study had multiple queens, eggs, larvae, and pupae. We marked 52 queens on the thorax or abdomen using unique color combinations of nail polish. Queens are easily distinguishable from workers by being 3 to 4 times larger (workers 1.2–1.5 mm long, queens 4.5–5 mm; Wetterer and Porter, 2003).

Behavioral Flexibility of Queens Assays

Six nests ($N = 6$) with a total of 19 queens were used for behavioral flexibility assays. Colonies were kept in the artificial nests 3 days prior to the start of experiment and fed daily. During the experiment observation period, queens were observed for 10 min daily for a total of 19 days; food was removed after each observation period. The 10 min observation period was determined after observing that the proportion of behaviors does not vary significantly in 10, 15, 20, and 30 min intervals.

TABLE 1 | Observation table.

Behavior flexibility assay observations	
Behaviors observed	**Number of extrapolated observations**
Egg Laying	2,204
Nursing	1,064
Foraging	54

Observations 10 min twice a day and extrapolated to 12 h periods for 19 days Number of total observations during behavioral assays and extrapolated to 12 h periods. Behaviors observed and tabulated were the number of events counted during the observation period.

Observations were made for different tasks, including egg laying (a typical behavior), nursing (i.e., manipulating brood) and foraging (i.e., queens seen feeding in the designated arena), the latter two being non-typical behaviors. During the 10 min observational period, we tabulated by number of events, i.e., number of eggs laid, number of times brood was manipulated, and number of times queens walked to the feeding arena and was seen feeding. Control observation periods were performed for a period of 5 days. After the first 5 days of observations, worker population per nest was estimated via nest pictures. We then randomly culled approximately 50% of workers from the nest to simulate natural events in the wild. Preliminary observations (Rivera-Marchand and Fernández-Casas unpublished) indicated that the worker caste is equally divided between nurses and foragers. Daily 10 min observations continued for 14 days (**Table 1**). The remaining nests were used to measure *JHbp* and *Vg* gene expression.

Bioinformatic Analysis and Primer Design

Primers were designed for gene sequences related to JH and Vg expression. Since JH is a terpenoid, its gene expression levels were determined indirectly by measuring Juvenile hormone binding protein (JHbp), an associated protein. JHbp is directly correlated with the onset of JH production in the hemolymph (Kramer et al., 1976; Shemshedini and Wilson, 1990) because it prevents the absorption and enzymatic hydrolysis of JH, thereby maintaining a steady reservoir of the hormone in the hemolymph. As a consequence, free JH is virtually absent (Roe and Venkatesh, 1990; Tan, 2007). Also, JHbp/JH interaction is specific and of high affinity (KD = 10^{-9}M), more than 99% of JH is bound to JHbp (De Kort and Granger, 1996; Tan, 2007). Other studies have further suggested this direct involvement as well (Prestwich et al., 1996; Hagai et al., 2007). Sequences for JHbp and Vg of *W. auropunctata* were obtained from NCBI Gene Bank. Vg sequences (XM_011697672.1, XM_011697673.1) were aligned using MAFFT (Multiple sequence alignment tool: Katoh et al., 2009). Primers (**Table 2**) were designed using primer3 from NCBI (Ye et al., 2012) with the obtained consensus sequence for Vg and the JHbp sequence (XM_011708554). Actin and GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase) were used as housekeeping genes (Wong and Medrano, 2005; Scharlaken et al., 2008).

RNA Extraction, cDNA and qPCR of *JHbp* and *Vg*

Fourteen nests (N = 14), different from the ones used in the first behavioral assay, were used with a total of 33 queens. Nests were randomly assigned to control or experimental groups (nest with workers removed), behavioral assays were repeated. Seven days after worker removal, queens were collected by tasks; from control nests n = 15 and from experimental nests n = 18 and placed in a microtube with 20 μL of RNAlater reagent (Qiagen, Valencia, CA, United States) stored at −80°C for later RNA extraction. Afterward, samples were placed in a sterilized microtube and mechanically homogenized. RNA extraction was performed using the RNeasy Mini Kit (Qiagen). Extracted RNA was quantified for each sample in μg/μL units using a Nanophotometer (Implen, Westlake Village, CA, United States). RNA was normalized to a concentration (10 μg/μL) in a final volume of 20 μL and treated with DNase 1, following BioLabs (Ipswich, MA, United States) protocol to remove any DNA contamination. cDNA was synthesized from the normalized RNA using iScript Reverse Transcription Supermix for RT-qPCR (Bio-Rad, Hercules, CA, United States) following the manufacturer's protocol with 10 μL of RNA as a template. cDNA synthesis was verified in an electrophoresis 1% ETBR-gel.

qPCR was performed using the MJ Mini-Opticon Real-Time PCR (Bio-Rad) following the standard protocol of forty cycles; denature at 95°C for 10 s, annealing at 56°C for 30 s and elongation at 72°C for 15 s × 40, with post-amplification melt curve analysis. As a standard for quantification purposes, actin and GAPDH were used as reference genes (Wong and Medrano, 2005; Scharlaken et al., 2008). Primer efficiency was calculated using the standard curve analysis method where 1 μl of each cDNA sample were pooled and serial diluted in five points at 1:10. Reactions were prepared with 2 μL of first strand cDNA as a template in a master mix of 1 μL of forward and reverse primers per gene at [10 nM] and 5 μL of iTaq Universal SYBR Green Supermix (Bio-Rad) in a final volume of 10 μL. Relative gene expression was calculated using the geometric mean

TABLE 2 | Primer table.

Gene	Acc. Number	Strand	Primer sequence	Amplification (bp)
JHbp	XM_011708554	FW	TGTTGGTGCCCATCGCTAAT	140
		RV	GTCCAGCTTTATCGTCAACTTCG	
Vg	XM_011697672.1, XM_011697673.1	FW	GCCACAACTGATCACAGCCA	218
		RV	GGACCGTCCGGTAATGTAGT	
		RV	GAGTTTGCTGCTGTGTTC	
Actin	AB023025	FW	TGCCAACACTGTCCTTTCTG	155
		RV	AGAATTGACCCACCAATCCA	
GAPDH	XM_393605	FW	GATGCACCCATGTTTGTTTG	203
		RV	TTTGCAGAAGGTGCATCAAC	

List of target genes including housekeeping genes primers, their accession number in NCBI and amplification length. Actin and GAPDH primer sequences were taken from Scharlaken et al., 2008.

analysis method (Vandesompele et al., 2002), using the following equation:

$$Relative\ gene\ expression = \frac{(E_{GOI})^{\Delta CtGOI}}{GeoMean}\left[(E_{REF})^{\Delta CtREF}\right],$$

E, primer efficiency; GOI, gene of interest; GeoMean, geometric mean; and REF, reference gene. ΔCt was calculated using the average Ct values of the control group for each gene (calibrator Ct). The relative expression values presented are relative to the control group.

Statistical Analysis

Behavior Analysis

For each task, relative probability was calculated by the number of queens performing a task with the total number of queens. Differences in relative probability between before and after worker removal were calculated with a Wilcoxon signed-rank test. To compare frequency of queen behaviors, frequency of typical and non-typical tasks were calculated by counting the number of events by queens before and after manipulation. Frequencies were compared using a Friedman test and Dunn's test as a *Post Hoc* method.

Gene Expression

In order to compare relative expression between control and experimental samples, a Wilcoxon signed-rank test was used to measure differences in expression of *JHbp* and *Vg*. Here experimental samples were considered queens performing both nursing and/or foraging. To verify relative gene expression of *JHbp* and *Vg* differences among all tasks (egg laying, nursing, and foraging), a Kruskal-Wallis rank sum test was used with Dunn's test as a *Post Hoc* method.

Data was analyzed using the statistical program R (R Core Team, 2014) v. 3.5.2 (2018-12-20) and the package agricolae (Statistical Procedures for Agricultural Research) v. 1.3-1. Graphs were done in Graph Pad Prism 6.0, (GraphPad Software, La Jolla, CA, United States). Data sets (Ortiz-Alvarado and Rivera-Marchand, 2020) can be found below https://datadryad.org/stash, https://doi.org/10.5061/dryad.j6q573nb2.

RESULTS

Behavior Analysis

In the first 3 days of observations queens laid eggs while workers performed typical tasks. After worker removal queens performed worker tasks for nine consecutive days, which coincided with pupae emergence. During the period after worker removal in which queens behaved as workers, egg laying decreased significantly (**Figure 1A**: $W = 62.5$, p-value = 0.01) while nursing and foraging behaviors increased significantly (**Figure 1B**: $W = 2.5$, p-value = 0.002; **Figure 1C**: $W = 15$, p-value = 0.04). Of note, during the behavior observation period, some of the queens in the nests remained idle.

Throughout the experiment when queens performed non-typical tasks, egg laying decreased until new workers emerged (**Figure 2**). After new workers emerged, queens

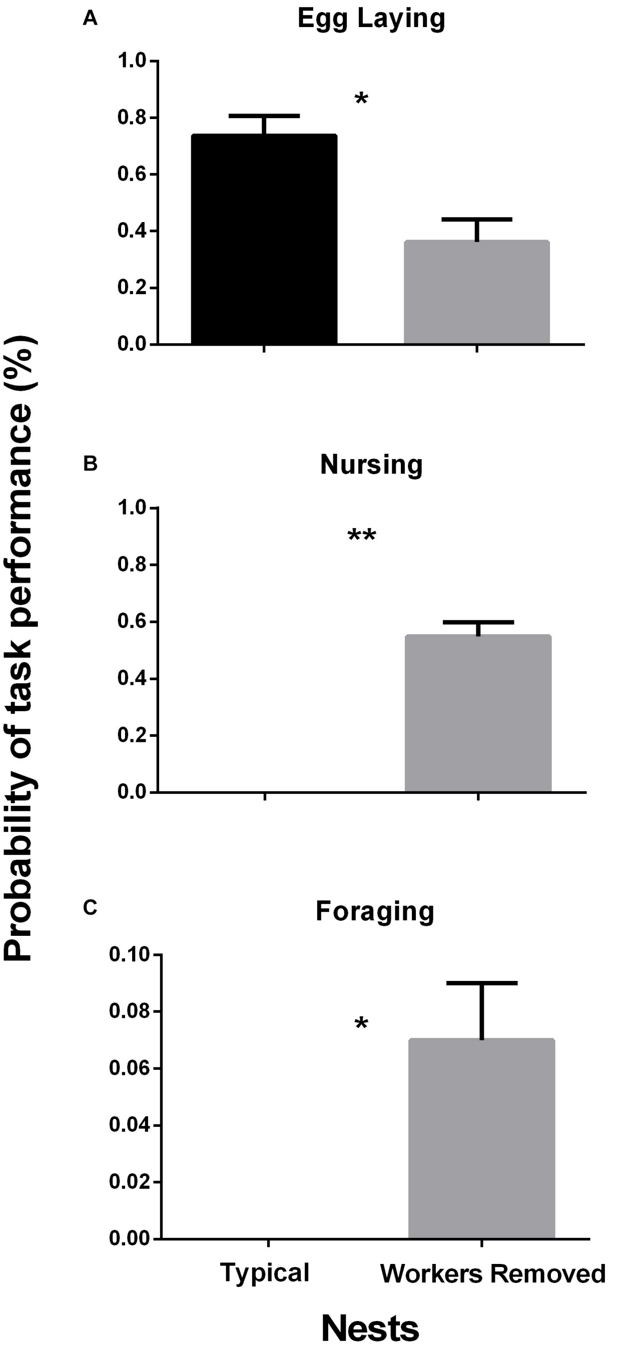

FIGURE 1 | Relative probability of tasks in egg laying behavior **(A)**, nursing behavior **(B)**, and foraging behavior **(C)**. **(A)** Egg Laying Behavior. Queen egg laying behaviors before and after workers were removed, task decreased significantly, $W = 62.5$, p-value = 0.01, $\text{Mean}_{\text{typical}} = 0.75$, $\text{SE}_{\text{typical}} = 0.07$, $\text{Mean}_{\text{WR}} = 0.36$, $\text{SE}_{\text{WR}} = 0.08$. **(B)** Nursing Behavior. Queen nursing behaviors before and after workers were removed, task increased significantly, $W = 2.5$, p-value = 0.002, $\text{Mean}_{\text{typical}} = 0.00$, $\text{SE}_{\text{typical}} = 0.00$, $\text{Mean}_{\text{WR}} = 0.55$, $\text{SE}_{\text{WR}} = 0.05$. **(C)** Foraging Behavior. Queen foraging behaviors before and after workers were removed, task increased significantly, $W = 15$, p-value = 0.04, $\text{Mean}_{\text{typical}} = 0.00$, $\text{SE}_{\text{typical}} = 0.00$, $\text{Mean}_{\text{WR}} = 0.07$, $\text{SE}_{\text{WR}} = 0.02$. $n = 19$ queens. Behaviors observed tabulated as number of events occurred during the observation period. Asterisks (*) = p-value of ≤ 0.05, (**) = p-value of ≤ 0.001.

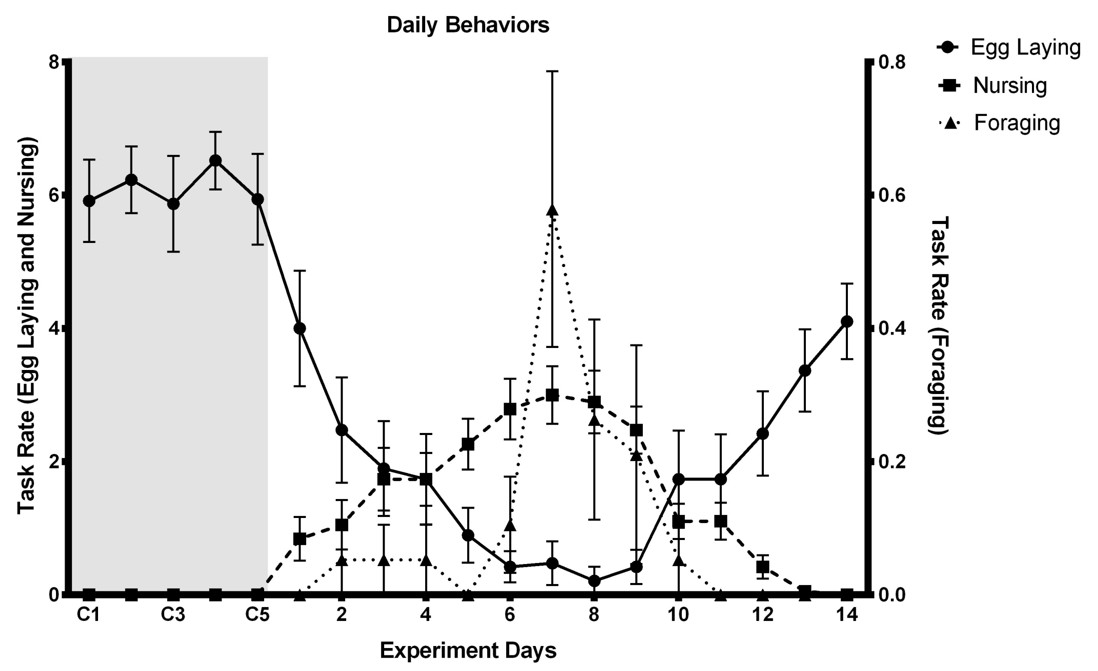

FIGURE 2 | Daily behavior rate. Task frequency of Queens (*n* = 19) during the experiment. The shaded area represents tasks during the control period (5 days). Workers were removed on day 5 after final control observations (C5). Egg laying behavior decreased significantly at day 4; Chi-square = 118.524, df = 18, *p*-value $\leq$ 0.001, just as nursing behavior increased significantly; Chi-square = 14.58, df = 18, *p*-value $\leq$ 0.001, foraging increased significantly, at day 7; Chi-square = 37.71, df = 18, *p*-value $\leq$ 0.001. On day 12 the population began to grow as workers emerged. At day 13 queens increased egg laying and decreased nursing, completing a cycle. First 3 days show no significant difference with the last 3 days; Dunn *p*-value $\geq$ 0.05. Behaviors were observed tabulated as number of events occurred during the observation period.

returned to egg laying and stopped performing worker tasks. *Post hoc* comparisons indicate that egg laying frequency from day 4 through 10 are significantly lower (*p*-value $<$ 0.05) than egg laying before worker removal. As egg laying decreased, the frequency of nursing behavior by queens increased significantly (*p*-value $<$ 0.05) from the fourth through tenth day of observations. While performing nursing, queens were seen manipulating eggs, larvae and pupae. Queens (*N* = 6) were also seen foraging during days 6 through 9. Only on day 7 was foraging frequency significantly higher than the rest of the experiment. On day 11 the queens returned to egg laying and by day fourteen queen behaviors resembled the behaviors during control period (*p*-value $>$ 0.05), thus showing that the nests endured the experimental period and returned to a typical behavioral pattern.

Gene Expression

There's a significant difference between the control and the experimental samples for *JHbp* and *Vg* expression. Queens from experimental nests performing worker related tasks have a lower relative gene expression of *JHbp* and *Vg* related to control nest queens (**Figure 3A**: *W* = 270, *p*-value $\leq$ 0.001; **Figure 3C**: *W* = 270, *p*-value $\leq$ 0.001). When relative gene expression of *JHbp* and *Vg* were compared between tasks, it shows a difference between egg laying compared to nursing and foraging for both *JHbp* and *Vg*; however, there is no difference in relative gene expression of *JHbp* and *Vg* in queens performing nursing or foraging tasks (**Figure 3B**:, Kruskal-Wallis chi-squared = 24.21, df = 2, *p*-value $\leq$ 0.001; **Figure 3D**; Kruskal-Wallis chi-squared = 25.19, df = 2, *p*-value $\leq$ 0.001).

DISCUSSION

Although behavioral flexibility is known to be a trait of workers in many species of social Hymenoptera (Gordon, 1991; Robinson, 1992; Giray et al., 2005; Seid and Traniello, 2006), queens of the little fire ant also demonstrate behavioral flexibility. When worker population decreases they perform worker tasks, principally nursing and, with less frequency, foraging (**Figure 2**). Although both behaviors were not initially expected, foraging in particular was a surprising outcome. Both egg laying and nursing are tasks done within the nest, so we hypothesize that the reason queens shift primarily to nursing is that it is less risky. Queens are risk aversive, and tend to perform nursing rather than foraging because the former is a less risky worker task. To our knowledge, this is the first time queens have been observed demonstrating behavioral flexibility after the founding stage. Given that *W. auropunctata* queens never experience a solitary founding stage (Foucaud et al., 2006; Mikheyev et al., 2009) and thus under typical conditions never perform worker-like duties, our results are even more remarkable.

During the experiment, there was an apparent transition phase, where queens gradually decreased their egg laying while

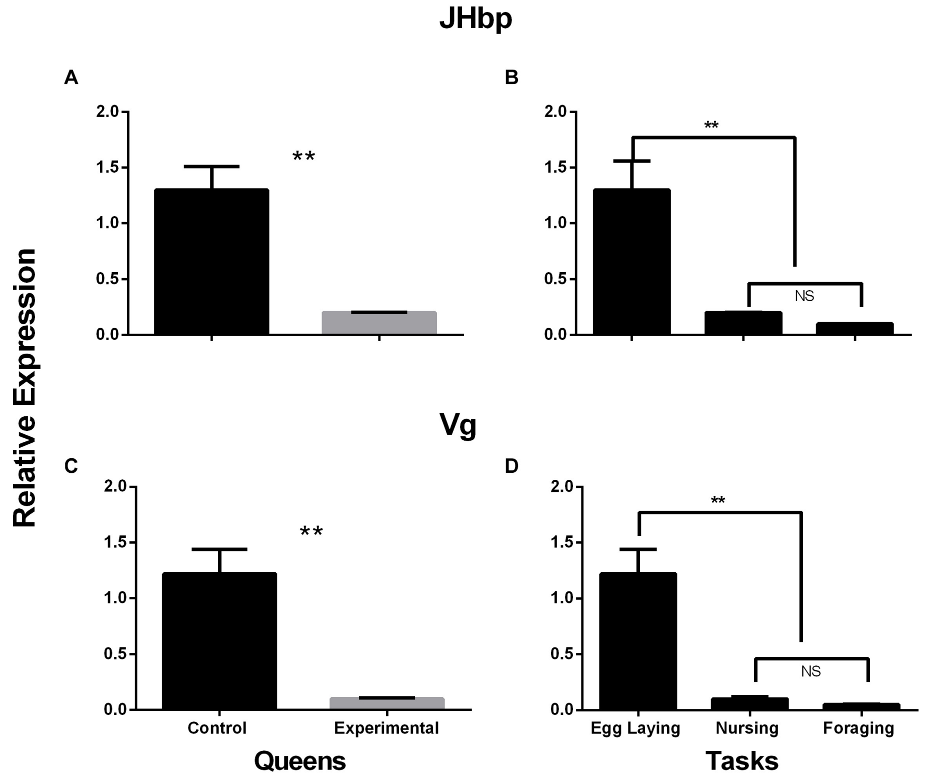

FIGURE 3 | Relative gene expression of JHbp **(A,B)** and Vg **(C,D)** in control and experimental nests. Results show differences in gene expression of JHbp and Vg between control or egg laying queens and experimental or nursing and foraging queens. Expression was measured in relative expression to endogenous genes Actin and GAPDH by geometric mean analysis method. $n = 33$ queens; control queens $n = 15$, experimental queens $n = 18$. **(A)** JHbp expression decreases from control to experimental queens, $W = 270$, p-value ≤ 0.001, $\text{Mean}_{\text{control}} = 1.30$, $\text{SE}_{\text{exp}} = 0.26$. **(B)** Comparing JHbp expression by tasks shows difference between egg laying compared to nursing and foraging, there's no difference in expression between nursing or foraging queens Kruskal-Wallis chi-squared = 24.21, df = 2, p-value ≤ 0.001, $\text{Mean}_{\text{egg laying}} = 1.30$, $\text{SE}_{\text{egg laying}} = 0.26$, $\text{Mean}_{\text{nursing}} = 0.20$, $\text{SE}_{\text{nursing}} = 0.002$, $\text{Mean}_{\text{foraging}} = 0.10$, $\text{SE}_{\text{egg laying}} = 0.003$. **(C,D)**. Similar pattern as JHbp is seen with Vg expression, decreases in experimental queens ($W = 270$, p-value ≤ 0.001; Kruskal-Wallis chi-squared = 25.19, df = 2, p-value ≤ 0.001, $\text{Mean}_{\text{control}} = 1.22$, $\text{SE}_{\text{exp}} = 0.22$, $\text{Mean}_{\text{egg laying}} = 1.22$, $\text{SE}_{\text{egg laying}} = 0.22$, $\text{Mean}_{\text{nursing}} = 0.10$, $\text{SE}_{\text{nursing}} = 0.02$, $\text{Mean}_{\text{foraging}} = 0.05$, $\text{SE}_{\text{egg laying}} = 0.01$; **(C,D)**, respectively. Asterisks (**) = p-value of ≤ 0.001.

increasing worker tasks. It is likely that queens may sense the shortage of workers due to a decrease in contact with workers. Studies have shown that ants typically communicate by cuticle hydrocarbons which are perceived by the olfactory organs (Vander Meer et al., 1989; Saïd et al., 2005; Ichinose and Lenoir, 2009; Bos et al., 2010), hence a decrease in the amount of cuticle hydrocarbons perceived might be an indicator to queens that the worker population has decreased. Maximum worker behavior frequencies were observed on the 8th day of the experiment, coinciding with minimum egg laying frequencies. We observed individual queens performing both nursing and foraging tasks. After the 9th day an increase in egg laying and a decrease in nursing were observed. From day 6 to 9, we observed queens eating at the feeding arena. Queens seem to decrease investments in reproduction in order to invest in brood care. As adult workers emerge and take over brood care tasks, queens begin investing in egg laying again. Since energy expenditures due to reproduction tend to be high in social insects (Oster and Wilson, 1978), queens of the little fire ant may not be able to invest in both egg laying and brood care. It is possible that these queens are diverting energy typically used for egg production to carry other nest duties. In our study queens performing worker tasks were not observed laying eggs. Moreover, studies on the reproductive biology of *W. auropunctata* have shown that all queens within nests produce viable eggs (de Ulloa, 2003). Therefore, we worked under the assumption that queens in the experiment were inseminated.

The changes in behaviors of queens were associated with changes in the expression of *JHbp* gene and *Vg*. When queens are performing typical tasks, the levels of *JHbp* and *Vg* tend to be higher, than when a queen displays worker behaviors. This suggests regulation by molecular and physiological mechanisms on behaviors, such as regulation by hormones/protein through gene expression. In honey bees, queens tend to have low levels of JH (Robinson et al., 1991; Fahrbach et al., 1995), the opposite is seen in the little fire ant queens, where queens performing egg laying had higher gene expression of *JHbp* gene than those performing worker related tasks (**Figure 3A**). When studying Vg, this protein has been found to have an effect on reproductive division of labor on honey bees, where queens have higher levels of Vg which decrease in nurses and foragers (Nelson et al., 2007; Page and Amdam, 2007). This is not an exception in other insects such as *S. invicta* and *P. canadensis* (Brent and Vargo, 2003; Sumner et al., 2006). The same pattern has been found in *W. auropunctata* queens, where queens have higher expression levels of *Vg* when laying eggs compared to the expression levels of *Vg* in the performance of worker related tasks (**Figure 3B**). Furthermore, studies have shown precocious foraging in workers induced by downregulation Vg using RNAi (Nelson et al., 2007; Antonio et al., 2008), suggesting changes in behavior are mediated by Vg.

The relationship between JH and Vg has been studied before (Robinson and Vargo, 1997; Lewis et al., 2001; Brent and Vargo, 2003; Barchuk et al., 2004). In most insects, there is a positive relationship between JH and Vg; increasing levels of JH cause an increase in Vg synthesis (Barchuk et al., 2004; Toth and Robinson, 2007). In *S. invicta* queens, high levels of JH and Vg are correlated. JH has been found to be an important promoter of vitellogenic oogenesis (Brent and Vargo, 2003; Lu et al., 2009) therefore, a decrease of JH seems to interrupt egg production. Our results show a similar relation with JH (*JHbp*) and Vg as queens' reduction in Vg expression seems to correspond to a reduction in JHbp gene expression.

Behavioral flexibility in social insects ensures survival, particularly where environmental changes may affect colony demography. The adaptive value of behavioral flexibility in workers has been evidenced in honey bees (Robinson, 1992; Scheiner et al., 2004), different *Pheidole* species (Seid and Traniello, 2006; Mertl and Traniello, 2009) and the harvester ant (*Pogonomyrmex barbatus*; Gordon, 1991, 2002), where increased needs for one task due to environmental changes may lead to a behavioral response. Flexibility in queen behaviors in the little fire ant may be an important adaptation to changing environments. As a native to the tropics, this ant may face frequent disturbances that may deplete worker population. Moreover, the colonies of this ant tend to move their nests often (Wetterer and Porter, 2003). During the process of moving, workers such as foragers may be left behind as the nest is moved. The probability of colony survival may increase with queens performing worker duties as an adaptation for the population reduction that might occur during propagation. Evidence of this increased probability of survival can be seen in the final phase of the experiment where queens returned to their typical behaviors as the worker population increased. The results of this study not only present new insights in behavioral flexibility in social insects, but also increases our understanding of the success of this important invasive species. The little fire ant, is an important invasive species and agricultural pest that has colonized many areas around the world. Various ecological and reproductive adaptations are associated to its success and the unique queen behavior of this study adds to our knowledge of the suite of adaptations allowing this ant to be a successful invader.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://datadryad.org/stash and https://doi.org/10.5061/dryad.j6q573nb2.

AUTHOR CONTRIBUTIONS

YO-A and BR-M conceived and designed the experiments presented in this manuscript, performed the writing of this manuscript, preparation of the figures, and editing. YO-A performed all of the experiments were in Puerto Rico and performed the data analysis on behavior and gene expression under the supervision of BR-M. All authors contributed to the article and approved the submitted version.

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REFERENCES

Amdam, G. V., Norberg, K., Hagen, A., and Omholt, S. W. (2003). Social exploitation of vitellogenin. *Proc. Natl. Acad. Sci. U.S.A.* 100, 1799–1802. doi: 10.1073/pnas.0333979100

Amsalem, E., Malka, O., Grozinger, C., and Hefetz, A. (2014). Exploring the role of juvenile hormone and vitellogenin in reproduction and social behavior in bumble bees. *BMC Evol. Biol.* 14:45. doi: 10.1186/1471-2148-14-45

Antonio, D. S. M., Guidugli-Lazzarini, K. R., Do Nascimento, A. M., Simões, Z. L. P., and Hartfelder, K. (2008). RNAi-mediated silencing of vitellogenin gene function turns honeybee (*Apis mellifera*) workers into extremely precocious foragers. *Naturwissenschaften* 95, 953–961. doi: 10.1007/s00114-008-0413-9

Azevedo, D. O., de Paula, S. O., Zanuncio, J. C., Martinez, L. C., and Serrão, J. E. (2016). Juvenile hormone downregulates vitellogenin production in *Ectatomma tuberculatum* (Hymenoptera: Formicidae) sterile workers. *J. Exper. Biol.* 219, 103–108. doi: 10.1242/jeb.127712

Barchuk, A. R., Maleszka, R., and Simões, Z. L. P. (2004). *Apis mellifera* ultraspiracle: cDNA sequence and rapid up-regulation by juvenile hormone. *Insect Mol. Biol.* 13, 459–467. doi: 10.1111/j.0962-1075.2004.00506.x

Bloch, G., Wheeler, D. E. and Robinson, G. E. (2002). *Endocrine Influences on the Organization of Insect Societies* in "Hormones, Brain, and Behavior", Vol. 3, eds D. W. Pfaff, A. P. Arnold, A. M. Ettgen, S. E. Fahrbach, and R. T. Rubin (San Diego: Academic Press), 195–237.

Bos, N., Guerrieri, F. J., and d'Ettorre, P. (2010). Significance of chemical recognition cues is context dependent in ants. *Anim. Behav.* 80, 839–844. doi: 10.1016/j.anbehav.2010.08.001

Brent, C. S., and Vargo, E. L. (2003). Changes in juvenile hormone biosynthetic rate and whole body content in maturing virgin queens of *Solenopsis invicta*. *J. Insect Physiol.* 49, 967–974. doi: 10.1016/s0022-1910(03)00166-5

Calabi, P., and Traniello, J. F. (1989). Behavioral flexibility in age castes of the ant *Pheidole dentata*. *J. Insect Behav.* 2, 663–677. doi: 10.1007/bf01065785

Corona, M., Libbrecht, R., Wurm, Y., Riba-Grognuz, O., Studer, R. A., and Keller, L. (2013). Vitellogenin underwent subfunctionalization to acquire caste and behavioral specific expression in the harvester ant *Pogonomyrmex barbatus*. *PLoS Genet.* 9:1003730. doi: 10.1371/journal.pgen.1003730

Corona, M., Velarde, R. A., Remolina, S., Moran-Lauter, A., Wang, Y., Hughes, K. A., et al. (2007). Vitellogenin, juvenile hormone, insulin signaling, and queen honey bee longevity. *Proc. Natl. Acad. Sci. U.S.A.* 104, 7128–7133. doi: 10.1073/pnas.0701909104

De Kort, C. A. D., and Granger, N. A. (1996). Regulation of JH titers: the relevance of degradative enzymes and binding proteins. *Archiv. Insect Biochem. Physiol.* 33, 1–26. doi: 10.1002/(sici)1520-6327(1996)33:1<1::aid-arch1>3.0.co;2-2

de Ulloa, P. C. (2003). Biologia reproductiva de *Wasmannia auropunctata* (R.)(*H ymenoptera*: *formicidae*). *Rev. Acad. Colomb. Cienc. Exact. Físic. Nat.* 27, 441–448.

Dolezal, A. G., Brent, C. S., Gadau, J., Hölldobler, B., and Amdam, G. V. (2009). Endocrine physiology of the division of labour in *Pogonomyrmex californicus* founding queens. *Anim. Behav.* 77, 1005–1010. doi: 10.1016/j.anbehav.2009.01.010

Dolezal, A. G., Brent, C. S., Hölldobler, B., and Amdam, G. V. (2012). Worker division of labor and endocrine physiology are associated in the harvester ant, *Pogonomyrmex californicus*. *J. Exper. Biol.* 215, 454–460. doi: 10.1242/jeb.060822

Dong, S. Z., Ye, G. Y., Guo, J. Y., and Hu, C. (2009). Roles of ecdysteroid and juvenile hormone in vitellogenesis in an endoparasitic wasp, *Pteromalus puparum* (Hymenoptera: Pteromalidae). *Gen. Comparat. Endocrinol.* 160, 102–108. doi: 10.1016/j.ygcen.2008.11.007

Fahrbach, S. E., Giray, T., and Robinson, G. E. (1995). Volume changes in the mushroom bodies of adult honey bee queens. *Neurobiol. Learn. Mem.* 63, 181–191. doi: 10.1006/nlme.1995.1019

Foucaud, J., Jourdan, H., Breton, J. L., Loiseau, A., Konghouleux, D., and Estoup, A. (2006). Rare sexual reproduction events in the clonal reproduction system of introduced populations of the little fire ant. *Evolution* 60, 1646–1657. doi: 10.1111/j.0014-3820.2006.tb00509.x

Giray, T., Giovanetti, M., and West-Eberhard, M. J. (2005). Juvenile hormone, reproduction, and worker behavior in the neotropical social wasp *Polistes canadensis*. *Proc. Natl. Acad. Sci. U.S.A.* 102, 3330–3335. doi: 10.1073/pnas.0409560102

Gordon, D. M. (1991). Variation and change in behavioral ecology. *Ecology* 72, 1196–1203. doi: 10.2307/1941093

Gordon, D. M. (1996). The organization of work in social insect colonies. *Nature* 380, 121–124. doi: 10.1038/380121a0

Gordon, D. M. (2002). The regulation of foraging activity in red harvester ant colonies. *Am. Nat.* 159, 509–518. doi: 10.1086/339461

Hagai, T., Cohen, M., and Bloch, G. (2007). Genes encoding putative Takeout/juvenile hormone binding proteins in the honeybee (Apis mellifera) and modulation by age and juvenile hormone of the takeout-like gene GB19811. *Insect Biochem. Mol. Biol.* 37, 689–701. doi: 10.1016/j.ibmb.2007.04.002

Hölldobler, B., and Wilson, E. O. (1990). *The Ants*. Cambridge MA: Harvard University Press.

Hölldobler, B., and Wilson, E. O. (1994). *Journey to The Ants*. Cambridge, MA: Harvard University Press.

Ichinose, K., and Lenoir, A. (2009). Ontogeny of hydrocarbon profiles in the ant *Aphaenogaster senilis* and effects of social isolation. *Compt. Rend. Biol.* 332, 697–703. doi: 10.1016/j.crvi.2009.04.002

Katoh, K., Asimenos, G., and Toh, H. (2009). Multiple alignment of DNA sequences with MAFFT. *Methods Mol. Biol.* 537, 39–64. doi: 10.1007/978-1-59745-251-9_3

Keller, L. (1991). Queen number, mode of colony founding, and queen reproductive success in ants (Hymenoptera Formicidae). *Ethol. Ecol. Evol.* 3, 307–316. doi: 10.1080/08927014.1991.9525359

Kramer, K. J., Dunn, P. E., Peterson, R. C., and Law, J. H. (1976). "Interaction of juvenile hormone with binding proteins in insect hemolymph," in *The Juvenile Hormones*, ed. L. I. Gilbert (Boston, MA: Springer), 327–341. doi: 10.1007/978-1-4684-7947-8_23

Le Breton, J., Chazeau, J., and Jourdan, H. (2003). Immediate impacts of invasion by *Wasmannia auropunctata* (Hymenoptera: Formicidae) on native litter ant fauna in a New caledonian rainforest. *Austral Ecol.* 28, 204–209. doi: 10.1046/j.1442-9993.2003.01266.x

Lewis, D. K., Campbell, J. Q., Sowa, S. M., Chen, M. E., Vinson, S. B., and Keeley, L. L. (2001). Characterization of vitellogenin in the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Apocrita: Formicidae). *J. Insect Physiol.* 47, 543–551. doi: 10.1016/s0022-1910(00)00155-4

Libbrecht, R., Corona, M., Wende, F., Azevedo, D. O., Serrão, J. E., and Keller, L. (2013). Interplay between insulin signaling, juvenile hormone, and vitellogenin regulates maternal effects on polyphenism in ants. *Proc. Natl. Acad. Sci. U.S.A.* 110, 11050–11055. doi: 10.1073/pnas.1221781110

Lu, H. L., Vinson, S. B., and Pietrantonio, P. V. (2009). Oocyte membrane localization of vitellogenin receptor coincides with queen flying age, and receptor silencing by RNAi disrupts egg formation in fire ant virgin queens. *FEBS J.* 276, 3110–3123. doi: 10.1111/j.1742-4658.2009.07029.x

Mertl, A. L., and Traniello, J. F. (2009). Behavioral evolution in the major worker subcaste of twig-nesting *Pheidole* (Hymenoptera: Formicidae): does morphological specialization influence task plasticity? *Behav. Ecol. Sociobiol.* 63, 1411–1426. doi: 10.1007/s00265-009-0797-3

Mikheyev, A. S., Bresson, S., and Conant, P. (2009). Single-queen introductions characterize regional and local invasions by the facultatively clonal little fire ant *Wasmannia auropunctata*. *Mol. Ecol.* 18, 2937–2944. doi: 10.1111/j.1365-294x.2009.04213.x

Mikheyev, A. S., Tchingnoumba, L., Henderson, A., and Alonso, A. (2008). Effect of propagule pressure on the establishment and spread of the little fire ant *Wasmannia auropunctata* in a Gabonese oilfield. *Diver. Distribut.* 14, 301–306. doi: 10.1111/j.1472-4642.2007.00463.x

Nelson, C. M., Ihle, K. E., Fondrk, M. K., Page, R. E., and Amdam, G. V. (2007). The gene vitellogenin has multiple coordinating effects on social organization. *PLoS Biol.* 5:e62. doi: 10.1371/journal.pgen.100062

O'Donnell, S. (2001). Worker age, ovary development, and temporal polyethism in the swarm-founding wasp *Polybia occidentalis* (Hymenoptera: Vespidae). *J. Insect Behav.* 14, 201–213.

Ortiz-Alvarado, Y., and Rivera-Marchand, B. (2020). *Data From: "Worker queens"? Behavioral Flexibility, Juvenile Hormone Binding Protein and Vitellogenin in Queens of the Little Fire Ant Wasmannia auropunctata*. Dryad: Dataset Publishing. Available online at: https://doi.org/10.5061/dryad.j6q573nb2

Oster, G. F., and Wilson, E. O. (1978). *Social Insects*. Princeton, NJ: Princeton University Press.

Page, R. E. Jr., and Amdam, G. V. (2007). The making of a social insect: developmental architectures of social design. *Bioessays* 29, 334–343. doi: 10.1002/bies.20549

Peeters, C., and Ito, F. (2001). Colony dispersal and the evolution of queen morphology in social Hymenoptera. *Annu. Rev. Entomol.* 46, 601–630.

Prestwich, G. D., Wojtasek, H., Lentz, A. J., and Rabinovich, J. M. (1996). Biochemistry of proteins that bind and metabolize juvenile hormones. *Archiv. Insect Biochem. Physiol.* 32, 407–419. doi: 10.1002/(sici)1520-6327(1996)32:3/4<407::aid-arch13>3.0.co;2-g

R Core Team (2014). *R: A Language And Environment For Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Robinson, G. E. (1992). Regulation of division of labor in insect societies. *Annu. Rev. Entomol.* 37, 637–665. doi: 10.1146/annurev.en.37.010192.003225

Robinson, G. E., Strambi, C., Strambi, A., and Feldlaufer, M. F. (1991). Comparison of juvenile hormone and ecdysteroid haemolymph titres in adult worker and queen honey bees (*Apis mellifera*). *J. Insect Physiol.* 37, 929–935. doi: 10.1016/0022-1910(91)90008-n

Robinson, G. E., and Vargo, E. L. (1997). Juvenile hormone in adult eusocial hymenoptera: gonadotropin and behavioral pacemaker. *Archiv. Insect Biochem. Physiol.* 35, 559–583. doi: 10.1002/(sici)1520-6327(1997)35:4<559::aid-arch13>3.0.co;2-9

Roe, R. M., and Venkatesh, K. (1990). Metabolism of juvenile hormones: degradation and titer regulation. *Morphogenet. Hormon. Arthrop.* 1, 126–179.

Rüppell, O., Schäffler, L., and Hölldobler, B. (2002). Lack of plasticity in the behavior of queens of the ant *Leptothorax rugatulus* emery (Formicidae: Hymenoptera). *J. Insect Behav.* 15, 447–454.

Saïd, I., Gaertner, C., Renou, M., and Rivault, C. (2005). Perception of cuticular hydrocarbons by the olfactory organs in *Periplaneta americana* (L.)(Insecta: Dictyoptera). *J. Insect Physiol.* 51, 1384–1389. doi: 10.1016/j.jinsphys.2005.09.001

Scharlaken, B., de Graaf, D. C., Goossens, K., Brunain, M., Peelman, L. J., and Jacobs, F. J. (2008). Reference gene selection for insect expression studies using quantitative real-time PCR: the head of the honeybee, *Apis mellifera*, after a bacterial challenge. *J. Insect Sci.* 8:33.

Scheiner, R., Page, R. E., and Erber, J. (2004). Sucrose responsiveness and behavioral plasticity in honey bees (*Apis mellifera*). *Apidologie* 35, 133–142. doi: 10.1051/apido:2004001

Seid, M. A., and Traniello, J. F. (2006). Age-related repertoire expansion and division of labor in *Pheidole dentata* (Hymenoptera: Formicidae): a new perspective on temporal polyethism and behavioral plasticity in ants. *Behav. Ecol. Sociobiol.* 60, 631–644. doi: 10.1007/s00265-006-0207-z

Shemshedini, L., and Wilson, T. G. (1990). Resistance to juvenile hormone and an insect growth regulator in *Drosophila* is associated with an altered cytosolic juvenile hormone-binding protein. *Proc. Natl. Acad. Sci. U.S.A.* 87, 2072–2076. doi: 10.1073/pnas.87.6.2072

Sullivan, J. P., Jassim, O., Fahrbach, S. E., and Robinson, G. E. (2000). Juvenile hormone paces behavioral development in the adult worker honey bee. *Horm. Behav.* 37, 1–14. doi: 10.1006/hbeh.1999.1552

Sumner, S., Pereboom, J. J., and Jordan, W. C. (2006). Differential gene expression and phenotypic plasticity in behavioural castes of the primitively eusocial wasp, *Polistes canadensis*. *Proc. R. Soc. B Biol. Sci.* 273, 19–26. doi: 10.1098/rspb.2005.3291

Tan, S. H. (2007). *Stable Expression and Purification of Juvenile Hormone Binding Protein from Drosophila melanogaster Schneider line-2 cells*. Doctoral Dissertation, University of Wisconsin, Madison. Available online at: http://digital.library.wisc.edu/1793/8134

Toth, A. L., and Robinson, G. E. (2007). Evo-devo and the evolution of social behavior. *Trends Genet.* 23, 334–341. doi: 10.1016/j.tig.2007.05.001

Tschinkel, W. R. (2006). *The Fire Ants*. Cambridge MA: Harvard University Press.

Vander Meer, R. K., Saliwanchik, D., and Lavine, B. (1989). Temporal changes in colony cuticular hydrocarbon patterns of *Solenopsis invicta*. *J. Chem. Ecol.* 15, 2115–2125. doi: 10.1007/bf01207442

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., et al. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol.* 3:research34.

Vargo, E. L., and Laurel, M. (1994). Studies on the mode of action of a queen primer pheromone of the fire ant *Solenopsis invicta*. *J. Insect Physiol.* 40, 601–610. doi: 10.1016/0022-1910(94)90147-3

Wetterer, J. K., and Porter, S. D. (2003). *The Little Fire Ant, Wasmannia Auropunctata: Distribution, Impact And Control*. *Sociobiology* 42, 1–41.

Wilson, E. O. (1971). *The Insect Societies*. Cambridge MA: Harvard University Press.

Wong, M. L., and Medrano, J. F. (2005). Real-time PCR for mRNA quantitation. *Biotechniques* 39, 75–85. doi: 10.2144/05391rv01

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., and Madden, T. L. (2012). Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinform.* 13:134. doi: 10.1186/1471-2148-14-134

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Honey Bees in the Tropics Show Winter Bee-Like Longevity in Response to Seasonal Dearth and Brood Reduction

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Upon their first introduction to Americas in 1956, African honey bees (*Apis mellifera scutellata*) hybridized with the previously introduced and already established European honey bees (EHBs). The resulting Africanized honey bees (AHBs) have spread through the continental tropics of the Western Hemisphere. The expansion of AHB has been constrained in temperate climates generally thought to be because of a lack of key adaptations required for successful overwintering. A drastic increase in longevity during broodless periods is crucial to colony survival. In the temperate regions, honey bee colonies become broodless in winter. While tropical regions do not experience winters as temperate zones do, seasonal changes in the abundance of floral resources cause variation in brood levels throughout the year. Here we use an island population of AHB in Puerto Rico (gAHB) to test the capacity of tropical-adapted honey bees to alter their longevity in different seasons, as well as under brood manipulation. We found that worker longevity in the gAHB colonies increases in the wet season (maximum longevity ca. 88 days vs. 56 days) in response to dearth of floral resources. A more pronounced increase in longevity was observed in response to manipulative reduction of the amount of open brood (maximum longevity 154 days). In addition, long lived gAHB demonstrated the signature winter bee-like hypopharyngeal gland size (average acini diameter 100.8 ± 6.2 μm at 65 and 70 days of age, $N = 26$), intermediate between forager (88.7 ± 5.9 μm, $N = 24$) and nurse (129.5 ± 8.1 μm, $N = 24$) gland size. We showed that gAHBs do not lack the adaptation to alter their longevity seasonally, though the magnitude of changes is less intense than those observed in EHBs during temperate winters. This suggests that increased longevity in response to limited capacity to rear brood is a shared character of Africanized and European honey bees.

Keywords: honey bees, life-span, brood, tropics, seasonal dearth

INTRODUCTION

Present honey bee distribution spans every continent except Antarctica. However, the species originated in lower latitudes and is unlikely to have experienced temperate climates until major expansions into higher latitudes (Cridland et al., 2017). Temperate winters are marked by low temperatures and a lack of floral resources. While extreme low temperatures are not a part of the tropical climate, seasonal dwindling of floral resources is a shared feature between the two regions (Little et al., 1977). Honey bees differ from virtually all other insects in their overwintering strategy. Rather than undergoing dormancy, honey bees survive the winter in a metabolically and physically active state using the food stored earlier in spring and summer and generating heat inside the thermoregulatory cluster (Stabentheiner et al., 2003, reviewed in Döke et al., 2015). Beginning at the end of summer and early fall, worker bees with increased longevity (up to four times that of spring months) gradually appear in the colonies (Fluri et al., 1982). By mid-fall, long-lived workers constitute the majority of the hive population (Fukuda and Sekiguchi, 1966; Mattila et al., 2001). In addition to the greater longevity, winter bees are different from their sisters that were produced in spring in their hypopharyngeal gland size, juvenile hormone and vitellogenin levels, and hemolymph protein content (Fluri et al., 1982). As part of this strategy, colonies reduce brood production during the winter months which creates a need for the existing workers (produced in fall) to sustain the colony population into the next spring (McLellan, 1978). There are two possible evolutionary histories for the winter-adaptive traits: (1) There has been selection as populations radiated into the temperate climates, favoring new variants with winter-adaptive traits (i.e., adaptive evolution) or (2) existing traits utilized to cope with environmental stressors in the tropics were coopted (i.e., exaptation). Examining flexible longevity in the introduced tropical honey bee populations in Americas can provide evidence in support of one or both scenarios.

The AHB populations in the Western Hemisphere were originally introduced in Brazil in 1956 and have subsequently hybridized with EHB previously brought over from Europe in the 1600s (Pinto et al., 2007; Rivera-Marchand et al., 2008; Kono et al., 2015; Lin et al., 2018). In an earlier study, it is reported that AHB in Venezuela demonstrated change in brood production over the tropical seasonal cycles suggesting tropical bees exhibit some degree of seasonal response (Winston et al., 2016). Here, we examined another tropical population of honey bees on the island of Puerto Rico (PR). The bees in PR are derived from Africanized honey bees, likely introduced from Texas ca. 1994 (Galindo-Cardona et al., 2013; Acevedo et al., 2019) and named gentle Africanized honey bees (gAHB) given a secondary, drastic reduction of colony defensive response (Rivera-Marchand et al., 2012; Avalos et al., 2017). In this study, we tested the capacity of gAHB to alter their longevity in accordance with different seasons and colony reproductive states in observational and experimental studies.

In PR, bees do not experience the same degree of cold stress that bees in temperate regions experience. Ambient temperatures in PR oscillate minimally both daily (maximum 8°C change within a day) and seasonally (maximum 3.5°C change between monthly average temperatures)[1]. However, despite relatively stable and warmer temperatures, PR has strong seasonal differences based on flower phenology (**Figure 1**). These differences in flower phenology create a similar stressor to temperate regions where forage resources are abundant in one part of the year and heavily reduced in the other. The decoupling of resource availability and cold stress, combined with the evolutionary history of the honey bees on the Island, makes PR an excellent location to test whether increased worker longevity is a winter-specific adaptation or a more generalized response to seasonal dearth.

While long-lived bees occur naturally in fall, their production can also be induced in summer by confining the queen in a cage inside the hive and thus preventing brood production (Maurizio, 1950; Fukuda and Sekiguchi, 1966; Fluri et al., 1982). In manipulative studies, the reduction in brood or open brood (i.e., larvae less than 5 days of age that require feeding by nurse bees) results in increased longevity (Maurizio, 1950, 1954; Eishchen et al., 1982; Omholt, 1988; Harbo, 1993; Amdam et al., 2009; Smedal et al., 2009; Döke et al., 2015). However, the studies demonstrating that limiting brood production generated workers displaying winter physiology were conducted with colonies from populations of EHB known to overwinter successfully in the temperate regions. Changes in longevity of tropical bees, either seasonally or induced by brood manipulations, have not been studied before.

In this study, we followed paint marked cohorts of worker honey bees within the colonies in our field station in PR to establish a baseline for the seasonal change in longevity in a tropical setting. We next tested the effect of experimentally induced reduction in brood availability, and its effect on traits generally considered winter-specific. We hypothesize that the presence of changes in winter-specific traits in a population that has been historically exempt from the burden of overwintering would suggest these are general adaptations induced by environmental stressors. Alternately, if no change is observed in gAHB, then adaptations must have evolved during the radiation to temperate regions and are maintained by selective pressure specific to winter conditions.

MATERIALS AND METHODS

Sources of Bees

All experimental gAHB colonies were maintained according standard techniques (Kevan, 2007) in the apiary located at the Agricultural Experimental Station of the University of PR Mayaguez in Gurabo, PR (N 18.257294, W 65.987871).

Seasonal Change in Worker Longevity

In October, 2018, we set up two healthy colonies, each with 10 frames of worker bees (~20 k bees), pollen, honey, capped and uncapped brood and a mated, laying queen. One of the colonies was selected as a donor for all the emerging adult bees to be marked and introduced into both colonies. Using a single source for the introduced workers provides a uniform genetic background for the subjects and thus limiting simplifying the experimental design compared to having two separate genetic backgrounds included as an independent variable. In the beginning of October, frames of capped brood ready to emerge were selected from each colony and placed in an incubator at 34°C with 60% relative humidity for approximately 24 h. One

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[1] https://www.weather.gov/sju/climo_pr_usvi_normals

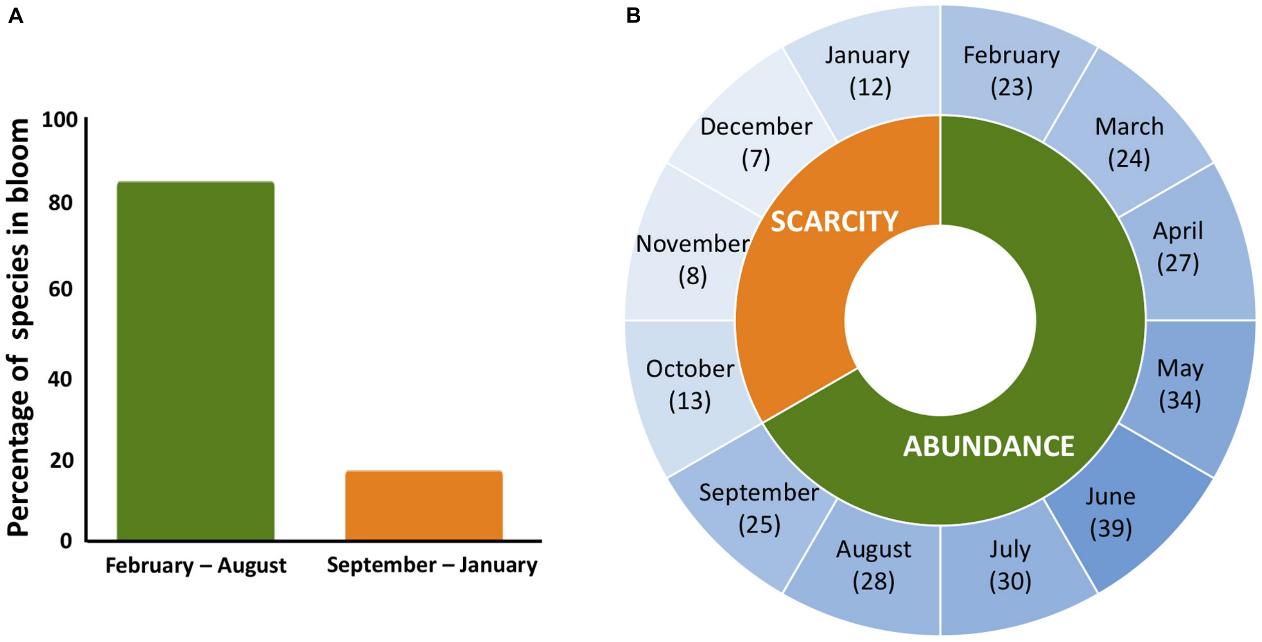

FIGURE 1 | Flower phenology in PR. **(A)** Blooming rate of the 61 trees in PR that are regularly visited by the honey bees throughout the year. **(B)** In PR, the number of species in bloom is highest in June and lowest in December. For 4 months (October–January) floral resources are scarce with an average of ten species in bloom compared to the other 8 months (February–September) when, on average, 29 species are flowering. Data from Little et al. (1977).

single cohort of 1,000 1-day old bees were paint marked on the thorax (Testors Paint, Rockford, IL) and 500 one-day-old bees were released into each colony. The number of marked survivors in each colony were recorded every 12 days until no more marked bees were observed in November. The counts were made by observing marked workers on frames and inner surfaces of the hive body early in the morning to avoid miscounting due to increased flight activity later in the day (Mattila et al., 2001).

Another set of longevity data collected from colonies set up in March 2018 is used for comparing worker longevity between seasons (see below for details). We used the data from control colonies which are unmanipulated (i.e., have mated, laying queens instead of caged queens). These were also the control colonies in the experiment testing the effect of elimination of open brood on worker longevity. Please note that we decided to report our results out of chronological order and rather based on a logical order for more effectively communicating the findings from separate and their relation to each other.

In October 2018, average temperature for the month was 27.7°C with a high of 32.8°C and low of 23.9°C; whereas, average temperature in March 2018 was 25.9°C with a high of 32.2°C and a low of 21.1°C[2].

Worker Longevity in Colonies With Curtailed Open Brood

In mid-March, 2018, 10 nucleus colonies each with 5 deep frames were established from existing full-size colonies in our research apiary. Control colonies (N = 5) composed of 1 empty frame with foundation, 1 frame of honey, 1 frame of pollen, and 2 frames of open brood and treatment colonies (N = 5) composed of 1 empty frame with foundation, 1 frame of honey, 1 frame of pollen, and 2 frames of capped brood for treatment colonies (see **Figure 2**, Step 1). Control colonies had laying queens while in treatment colonies, the queen was separated from workers via a push-cage (Maurizio, 1954; Fluri et al., 1982). The push-cage for queen confinement was made using wire mesh as in Le Conte et al. (2001) and this separation of the worker and queen breaks the brood production as the small number of eggs laid by the queen cannot be cared for by nurse bees. At the point of establishment all five frames in each colony were fully covered in worker bees bringing the colony population to an estimated 15000 workers (Jeffree, 1951). Upon establishment of the nucleus colonies, frames of capped brood that are ready to emerge were collected from several full-size colonies in the same apiary and placed in an incubator at 34°C with 60% relative humidity for approximately 24 h (see **Figure 2**, Step 2). Over the next 2 days, 4000 1-day-old bees were paint-marked on the thorax (Testors Paint, Rockford, IL) and then 400 bees were introduced to each of 10 colonies to establish single cohort of bees of known age in each colony (Mattila et al., 2001, See **Figure 2**, Step 3). The treatment colonies were monitored regularly to ensure the queens were alive in the cage (Smedal et al., 2009). Colonies which became queenless (4 out of 10) were removed from the study and not included in the analyses even when data was collected prior to their removal. Periodical survival checks were made every 2 weeks from April until there were no marked survivors in the colonies (see **Figure 2**, Step 4). The counts were made early in the morning to avoid

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[2] https://w2.weather.gov/climate/index.php?wfo=sju

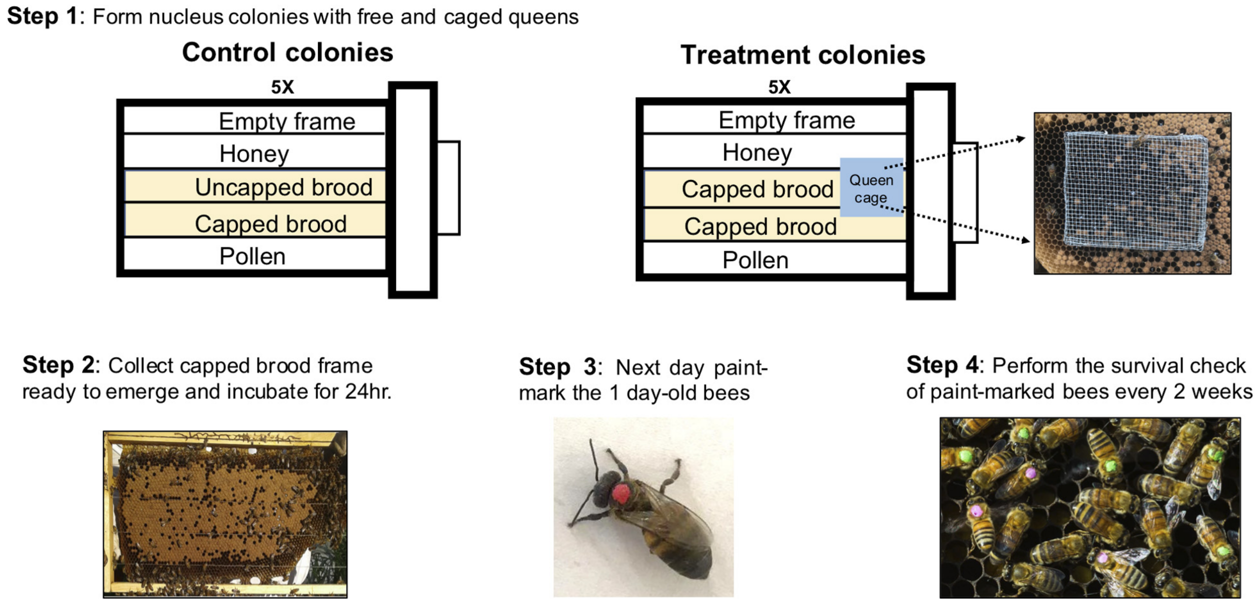

FIGURE 2 | Overview of the overall strategy, materials, and methods for worker life-span with reduced brood rearing. Control Colonies (CC) were set up to approximate the typical conditions of a colony in spring. Although no uncapped brood was present in Treatment Colonies (TC), overall amount of brood was equalized between the CC and TC by introducing capped brood from CC to TC as needed.

miscounting due to increased flight activity later in the day (Mattila et al., 2001).

Sample Collection

Worker honey bees were collected from both control and treatment (curtailed brood) colonies that were established in March 2018. Twelve days after the introduction of marked worker bees, nurses were collected via behavioral observations of brood care, while the bees were feeding the young brood (as in Vannette et al., 2015). Twenty-one days after the introduction of marked worker bees, foragers were collected by temporarily blocking the hive entrance using a wire mesh and individually picking the workers coming back from foraging flights with a visible load of pollen (as in Giray et al., 1999; Vannette et al., 2015). Later samples were collected from inside the nucleus colonies merely based on marked worker age (45, 65, and 70 days old). All samples were collected into liquid nitrogen in the field, transported to the laboratory on dry ice in an insulated box, and kept at −80°C until processing (Grozinger et al., 2003).

Dissections and Hypopharyngeal Gland Measurements

Four bees per colony (age and treatment) were dissected. Frozen samples were kept on dry ice throughout their handling and the hypopharyngeal gland (HPG) was removed under a dissection microscope (Nikon C-LEDS, produced Nikon Instruments Inc., NY, United States). Dissected HPGs were kept in RNA*later* ICE Frozen Tissue Transition Solution (Thermo Fisher Scientific, MA, United States) until measurement of the acini diameter as an estimate of the HPG size (Renzi et al., 2016). The diameter of one acinus per sample was digitally measured in μm (Nikon Eclipse E200 microscope connected to a Nikon DS-Fi2 camera, Nikon Instruments Inc., NY, United States), then the mean acini size was calculated for each group. While it is common practice to use multiple acini measurements from each individual, the coefficient of variation within individual was small enough (0.09–0.21) to use single acinus data per worker with confidence to compare groups of workers (Shechtman, 2013).

Statistical Analyses

Kaplan-Meier Survival Analysis (Goel et al., 2009) was employed to compare the longevity of both cohorts of worker bees from different times in the year and cohorts from the manipulated versus control colonies. ANOVA was used to compare the acini size of worker bees of differing age that were collected from the experimental colonies. All statistical analyses were completed using JMP (JMP®, Version 14. SAS Institute Inc., Cary, NC, 1989–2019) and graphs were plotted using Prism (GraphPad Prism version 6.00 for Mac OS X, GraphPad Software, La Jolla, CA, United States). Additionally, Microsoft® PowerPoint® 2016 and an open access vector graphics software, INKSCAPE (Version 0.92) were used in image preparation.

RESULTS

Seasonal Change in Worker Longevity

Mean and standard deviation of longevity for worker bees in October cohort (1000 individuals from 2 colonies) was 26.8 ± 22.1 days, significantly longer than the 23.6 ± 12.9 days for the March cohort (2,000 individuals from 5 colonies) (Kaplan-Meier Survival Analysis, Log-Rank $p << 0.05$). None of the workers introduced to the colonies in March survived beyond 56 days while workers introduced in October had survivors up to 88 days (**Figure 3**). Note that the data represented as "March cohort" is the same as the data for control colonies (i.e., with mated, laying queen) in the manipulative experiment where half of the colonies had curtailed open brood. We chose to use this data for both comparisons because control colonies were non-manipulated and thus constitute a good representation of natural longevity for the season in which they were observed.

Worker Longevity in Colonies With Curtailed Brood Production

Maximum longevity in the treatment colonies was 154 days, while the bees of control colonies survived up to 56 days. Mean and standard deviation of longevity for worker bees released to colonies without open brood was 35.2 ± 24.5 days, significantly longer than the 23.6 ± 12.9 days for the workers in control colonies (Kaplan-Meier Survival Analysis Log-Rank $p << 0.05$, **Figure 4**). We also explored the effect of colony identity on longevity via comparing colonies within control and treatment groups and found a significant colony effect in each case (Kaplan-Meier Survival Analysis Log-Rank $p << 0.05$). Nevertheless, the range at which mean survival time in control colonies varied (19.7–26.9) was outside the range of mean survival in colonies with curtailed brood (31.0–39.3).

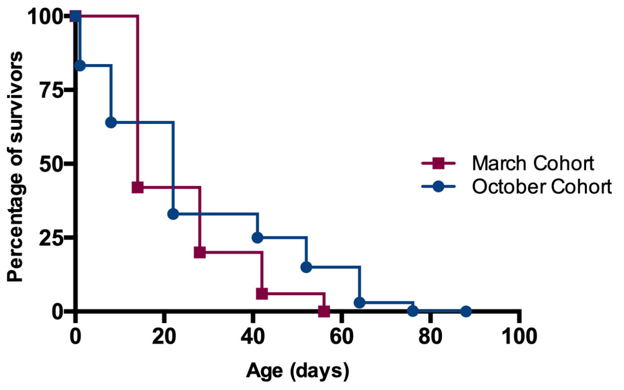

FIGURE 3 | Monitoring the longevity of worker bees in different months in PR. Cohort of 1-day old bees introduced in October had significantly greater longevity than the ones introduced in March (26.8 ± 0.7 days vs. 23.6 ± 0.3 days, Kaplan-Meier Survival Analysis Log-Rank $p << 0.05$).

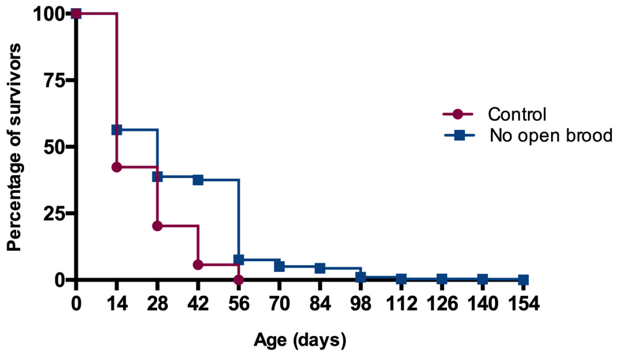

FIGURE 4 | Worker longevity is significantly higher in colonies with no open brood in comparison with control colonies (Kaplan-Meier Survival Analysis Log-Rank $p << 0.05$).

In both manipulated and natural colonies we observed large number of early losses of marked bees. Based on our observations, early deaths (noted at the first census) are due to the stress of handling and rejection by older workers in the recipient colonies. Early deaths in our data are in line with what was observed in introduced cohorts of bees in European honey bees in temperate regions (Mattila et al., 2001).

Acini Size of HPGs in Different Bee Types

As a reliable estimate of overall HPG size (Deseyn and Billen, 2005) acini diameter was measured for worker honey bees of different ages from control and treatment colonies. Acini diameter was significantly different across the bees of different age where 12-day-old nurses had the largest (129.5 ± 8.1 μm) and 21 day-old foragers had the smallest acini (88.7 ± 5.9 μm) (ANOVA, $p << 0.05$, **Figure 5**). Bees from treatment colonies exhibiting extreme longevity (65–75 days) had an intermediate acini size (100.8 ± 6.2 μm).

While the acini size for 12 day-old nurses was not different between the treatment and control colonies, there was a small but significant difference between the acini size of workers collected at the age of 21 and 45 days from manipulated and unmanipulated colonies. Note that there are no samples from control colonies after the 45-day time point because no workers survived to the next sampling day (65) in control colonies.

We explored the effect of colony environment on acini size via a nested ANOVA where colonies were nested within age groups and found that colony environment accounts for 14% of the total

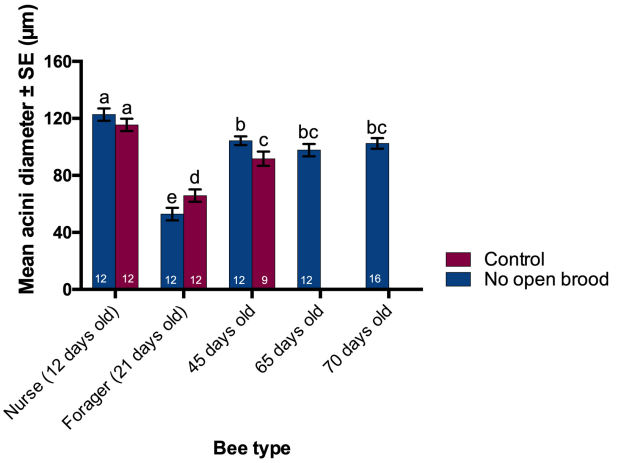

FIGURE 5 | Acini size of different bees collected at different times from treatment and control colonies. Hypopharyngeal gland acini size were significantly different between groups; largest in nurses, smallest in foragers, and intermediate in the long-lived winter-like bees (ANOVA, $p << 0.05$). Different letters denote significant differences in acini size between groups (Student's *t*-Test, $p < 0.05$).

variation observed while the remaining 86% can be attributed to brood status.

DISCUSSION

Here, we show increased longevity under typical seasonal conditions and upon brood manipulation in a tropical-adapted honey bee. Worker honey bees in temperate regions live up to 60 days in foraging season while a subpopulation of workers produced at the end of summer survive routinely more than 200 days through the winter (Fukuda and Sekiguchi, 1966; Southwick, 1991; Mattila et al., 2001). In this study, average worker longevity was significantly higher in the dearth season compared to when resources are ample. The direction of change in worker longevity was similar to reported for the honey bees in temperate regions, though less pronounced. By eliminating open brood, younger larvae that are fed by nurse bees and in cells that are not sealed with silk and wax, the gAHB workers were "winterized" and lived up to 5 months in spring and summer months.

One caveat of our study is that the longevity data collected in October came from worker bees harvested from a single colony and placed into two colonies upon marking them. Thus, all individuals involved are daughters of the same queen. However, naturally mated queen honey bees provide a large level of genetic variation because each queen mates with multiple drones (Kraus et al., 2005; Withrow and Tarpy, 2018). While not removing the influence of genetics on longevity, this approach provides a uniform genetic base instead of introducing various genetic make ups to the experimental design as an additional variable. Our results from the two independent colonies where open brood was eliminated via caging the queen and findings from earlier research in the temperate regions suggest that increased worker longevity in response to decreased brood is a general response in honey bees. In the future, experiments comparing various genetic backgrounds under brood-manipulated conditions as well as in different seasons could properly examine the interaction of genes with the signals for the seasonal change in worker longevity.

We also examined the HPGs of gAHBs. Size of HPG changes with the age and task of the bee in the hive, reflecting the behavioral status of the bee (Smodiš Škerl and Gregorc, 2015). Brood pheromone (BP) acts as a primer pheromone and facilitates the protein synthesis in the HPGs of workers (Brouwers, 1983; Huang and Otis, 1989; DeGrandi-Hoffman et al., 2010), which explains the largest HPG size of nurse bees. Interestingly, workers in winter have larger HPGs than summer foragers despite the lack of open brood (Moritz and Crailsheim, 1987; Hrassnigg and Crailsheim, 1998; Smodiš Škerl and Gregorc, 2015), suggesting a more complex effect of BP and interactions with brood on worker HPG size. The mean HPG size of the long-lived workers in gAHB colonies without open brood was larger than that of foragers and smaller than that of nurses as was previously shown for long-lived European honey bees in temperate regions (Fluri et al., 1982).

When taken together, the change in longevity and HPG size in the workers in colonies without open brood during floral abundance as in the March experiment, suggest that external seasonal conditions are indirectly communicated to the members through the change in the brood rearing capacity of the colony. Therefore, reduction in brood constitutes a signal for the existing workers to alter their biology in an adaptive manner in order to survive longer than usual through the dearth season. Previous studies in the temperate region show a gradual increase in the ratio of winter bees within the overall colony population corresponding to a gradual decrease in the brood rearing (Mattila et al., 2001; Mattila and Otis, 2007). This gradual change in proportion of long-lived bees implies the existence of a threshold effect where some workers respond to the brood signal earlier than the others. We have not examined systematically the change in brood production in PR honey bee colonies over the year. However, colony records for the amount of brood measured as combs with brood cataloged during the regular inspection of the hives by our field technician provides preliminary support. We present in Supplementary Material Appendix A records for 11 hives between February 2015 and January 2016 (number of hives were dictated by continuity). There was a significant effect of month of the year on the number of frames of brood [$F(17,179) = 1.78$, $p < 0.05$], with the lowest brood levels between the months of August and January (4.45 ± 0.12 frames), and highest between the months of February and July (5,47 ± 0.19 frames. These periods correspond, respectively, to presence of scarce versus abundant forage (Little et al., 1977 and **Figure 1**). Additional ambient cues such as daily cycles in temperature, light, humidity may be functioning as predictive *zeitgebers* alongside the brood signal (Frisch and Aschoff, 1987; Giannoni-Guzman et al., 2014; Moore, 2016). Nevertheless, the fact that simply removing open brood in gAHB colonies in PR in spring affects the life-span of worker bees shows that brood availability is a key component of the signal for seasonal change in workers physiology.

In gAHB of PR the change in worker longevity in response to reduced open brood is similar to what was observed in overwintering EHB colonies in the temperate regions. The gAHBs are an introduction from an Africanized population that likely originally from Texas ca. 1994 (Acevedo et al., 2019). In general, AHBs are reportedly less successful than EHBs in overwintering (Villa and Rinderer, 1993). The leading hypothesis for this difference is that Africanized honey bees could be lacking in flexibility to produce longer-lived winter workers which is critical for keeping the colony population alive when brood is reduced (Southwick, 1991). However, African honey bees routinely survive broodless conditions as a part of their life cycle (McNally and Schneider, 1992). While European honey bees stop or severely limit brood rearing in at least part of the winter, African honey bees become broodless for 4 to 6 weeks during migration (Grozinger et al., 2014). Nevertheless, AHBs have exhibited limited success in expanding into temperate climates in spite of their remarkably rapid spread elsewhere (Kono et al., 2015; Lin et al., 2018) suggesting that flexibility in worker longevity is necessary but certainly not sufficient for honey bee colonies to survive through the temperate winter. Successful colonies must also act proactively to perceive the change in seasons and accumulate floral resources that will sustain them through dearth (Seeley and Visscher, 1985; Döke et al., 2015, 2018) unlike their tropical counterparts who still have access to a greatly reduced but still present number of flowering plants (Little et al., 1977). Moreover, colonies in temperate regions must successfully thermoregulate to keep the surface of the winter cluster above the cold-paralysis threshold of 10–12°C against extreme ambient temperatures (Seeley, 1989; Southwick, 1991; Döke et al., 2015). In case of gAHB in PR, there is evidence that this population has been under selection, likely to increase hoarding in expense of colony defense (Rivera-Marchand et al., 2012; Avalos et al., 2017). This could place the gAHB population at an advantage over other AHBs in the mainland for expansion further into the temperate zones.

Lack of open brood constituting a reliable signal for the shift in worker longevity in both temperate and tropical settings in both European and Africanized honey bees suggests the existence of conserved biochemical and neurological mechanisms to asses and react to seasonal change. These mechanisms could have been acquired in an earlier stage of honey bee evolutionary history, prior to radiating to colder parts of the globe. There may have been further selection on each of these traits at various levels in different populations where colonies are faced with specific stressors of temperate winters versus tropical dearth. Genomic comparisons between the bee stocks of tropics and temperate regions can provide valuable information on the parts of the genome under selection for specific stressors.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

MD, CG, and TG designed and planned the study. MD, S-FC, and JA carried out the experiments. MD, S-FC, and JLA-R completed statistical analyses. MD, S-FC, JLA-R, CG, and TG prepared the manuscript for publication. All authors contributed to the article and approved the submitted version.

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REFERENCES

Acevedo, J. P., Alberto, G., Cardona, G., Whitfield, C. W., Rodriguez, D. M., Uribe, J. L., et al. (2019). Colonization history and population differentiation of the Honey Bees (*Apis mellifera* L.) in Puerto Rico. *Ecol. Evol.* 9, 10895–10902. doi: 10.1002/ece3.5330

Amdam, G. V., Rueppell, O., Fondrk, M. K., Page, R. E., and Nelson, C. M. (2009). The nurse's load: early-life exposure to brood-rearing affects behavior and lifespan in honey bees (*Apis mellifera*). *Exp. Gerontol.* 44, 467–471. doi: 10.1016/j.exger.2009.02.013

Avalos, A., Pan, H., Li, C., Acevedo-Gonzalez, J. P., Rendon, G., Fields, C. J., et al. (2017). A soft selective sweep during rapid evolution of gentle behaviour in an Africanized honeybee. *Nat. Commun.* 8:1550. doi: 10.1038/s41467-017-01800-0

Brouwers, E. V. M. (1983). Activation of the hypopharyngeal glands of honeybees in Winter. *J. Apic. Res.* 22, 137–141. doi: 10.1080/00218839.1983.11100576

Cridland, J. M., Tsutsui, N. D., and Ramírez, S. R. (2017). The complex demographic history and evolutionary origin of the western honey bee, *Apis Mellifera*. *Genome Biol. Evol.* 9, 457–472. doi: 10.1093/gbe/evx009

DeGrandi-Hoffman, G., Chen, Y., Huang, E., and Huang, M. H. (2010). The effect of diet on protein concentration, hypopharyngeal gland development and virus load in worker honey bees (*Apis mellifera* L.). *J. Insect Physiol.* 56, 1184–1191. doi: 10.1016/j.jinsphys.2010.03.017

Deseyn, J., and Billen, J. (2005). Age-dependent morphology and ultrastructure of the hypopharyngeal gland of *Apis mellifera* workers (Hymenoptera, Apidae). *Apidologie* 36, 49–57. doi: 10.1051/apido:2004068

Döke, M. A., Frazier, M., and Grozinger, C. M. (2015). Overwintering honey bees: biology and management. *Curr. Opin. Insect Sci.* 10, 185–193. doi: 10.1016/j.cois.2015.05.014

Döke, M. A., McGrady, C. M., Otieno, M., Grozinger, C. M., and Frazier, M. (2018). Colony size, rather than geographic origin of stocks, predicts overwintering success in honey bees (Hymenoptera: Apidae) in the Northeastern United States. *J. Econ. Entomol.* 112, 525–533. doi: 10.1093/jee/toy377

Eishchen, F. A., Rothenbuhler, W. C., and Kulinčević, J. M. (1982). Length of life and dry weight of worker honeybees reared in colonies with different worker-larva ratios. *J. Apic. Res.* 21, 19–25. doi: 10.1080/00218839.1982.11100511

Fluri, P., Lüscher, M., Wille, H., and Gerig, L. (1982). Changes in weight of the pharyngeal gland and haemolymph titres of juvenile hormone, protein and vitellogenin in worker honey bees. *J. Insect Physiol.* 28, 61–68. doi: 10.1016/0022-1910(82)90023-3

Frisch, B., and Aschoff, J. (1987). Circadian rhythms in honeybees: entrainment by feeding cycles. *Physiol. Entomol.* 12, 41–49. doi: 10.1111/j.1365-3032.1987.tb00722.x

Fukuda, H., and Sekiguchi, K. (1966). Seasonal change of the honeybee worker longevity in Sapporo, North Japan, with notes on some factors affecting the life-span. *Jpn. J. Ecol.* 16, 206–212.

Galindo-Cardona, A., Acevedo-Gonzalez, J. P., Rivera-Marchand, B., and Giray, T. (2013). Genetic structure of the gentle Africanized honey bee population (gAHB) in Puerto Rico. *BMC Genet.* 14:65. doi: 10.1186/1471-2156-14-65

Giannoni-Guzman, M. A., Avalos, A., Perez, J. M., Loperena, E. J. O., Kayım, M., Medina, J. A., et al. (2014). Measuring individual locomotor rhythms in honey bees, paper wasps and other similar-sized insects. *J. Exp. Biol.* 217, 1307–1315. doi: 10.1242/jeb.096180

Giray, T., Huang, Z. Y., Guzmán-Novoa, E., and Robinson, G. E. (1999). Physiological correlates of genetic variation for rate of behavioral development in the honeybee, *Apis mellifera*. *Behav. Ecol. Sociobiol.* 47, 17–28. doi: 10.1007/s002650050645

Goel, N., Banks, S., Mignot, E., and Dinges, D. F. (2009). PER3 polymorphism predicts cumulative sleep homeostatic but not neurobehavioral changes to chronic partial sleep deprivation. *PLoS One* 4:e5874. doi: 10.1371/journal.pone.0005874

Grozinger, C. M., Richards, J., and Mattila, H. R. (2014). From molecules to societies: mechanisms regulating swarming behavior in honey bees (Apis spp.). *Apidologie* 45, 327–346. doi: 10.1007/s13592-013-0253-2

Grozinger, C. M., Sharabash, N. M., Whitfield, C. W., and Robinson, G. E. (2003). Pheromone-mediated gene expression in the honey bee brain. *Proc. Natl. Acad. Sci. U.S.A.* 100, 14519–14525. doi: 10.1073/pnas.2335884100

Harbo, J. R. (1993). Effect of brood rearing on honey consumption and the survival of worker honey bees. *J. Apic. Res.* 32, 11–17. doi: 10.1080/00218839.1993.11101282

Hrassnigg, N., and Crailsheim, K. (1998). Adaptation of hypopharyngeal gland development to the brood status of honeybee (*Apis mellifera* L.) colonies. *J. Insect Physiol.* 44, 929–939. doi: 10.1016/S0022-1910(98)00058-4

Huang, Z. Y., and Otis, G. W. (1989). Factors determining hypopharyngeal gland activity of worker honey bees (*Apis mellifera* L.). *Insectes Sociaux* 36, 264–276. doi: 10.1007/BF02224880

Jeffree, E. P. (1951). A photographic presentation of estimated numbers of honeybees (*Apis Mellifera* L.) On Combs in 14 × 81/2 Inch Frames. *Bee World* 32, 89–91. doi: 10.1080/0005772x.1951.11094703

Kevan, P. G. (2007). *Bees: Biology and Management*. Enviroquest.

Kono, Y., Kohn, J. R., and Wicker-Thomas, C. (2015). Range and frequency of africanized honey bees in California (USA). *PLoS One* 10:e0137407. doi: 10.1371/journal.pone.0137407

Kraus, F. B., Neumann, P., and Moritz, R. F. A. (2005). Genetic variance of mating frequency in the honeybee (*Apis mellifera* L.). *Insectes Sociaux* 52, 1–5. doi: 10.1007/s00040-004-0766-9

Le Conte, Y., Mohammedi, A., and Robinson, G. E. (2001). Primer effects of a brood pheromone on honeybee behavioural development. *Proc. R. Soc. B Biol. Sci.* 268, 163–168. doi: 10.1098/rspb.2000.1345

Lin, W., McBroome, J., Rehman, M., and Johnson, B. R. (2018). Africanized bees extend their distribution in California. *PLoS One* 13:e0190604. doi: 10.1371/journal.pone.0190604

Little, E. L., Wadsworth, F. H., and Marrero, J. (1977). *Arboles Comunes de Puerto Rico y las Islas Virgenes (First)*. Available online at: http://edicionesdigitales.info/biblioteca/arbolesprvi1esp.pdf (accessed January 5, 2020).

Mattila, H. R., Harris, J. L., and Otis, G. W. (2001). Timing of production of winter bees in honey bee (*Apis mellifera*) colonies. *Insectes Sociaux* 48, 88–93. doi: 10.1007/PL00001764

Mattila, H. R., and Otis, G. W. (2007). Dwindling pollen resources trigger the transition to broodless populations of long-lived honeybees each autumn. *Ecol. Entomol.* 32, 496–505. doi: 10.1111/j.1365-2311.2007.00904.x

Maurizio, A. (1950). The influence of pollen feeding and brood rearing on the length of life and physiological condition of the honey bee. *Bee World* 31, 9–12.

Maurizio, A. (1954). Pollenernahrung und ILcbrnsvorgange bei der Honigbiene. *Landw. Jb. Schweiz.* 68, 115–182.

McLellan, A. (1978). Growth and decline of honeybee colonies and inter-relationships of adult bees, brood, honey and pollen. *J. Appl. Ecol.* 15, 155–161.

McNally, L. C., and Schneider, S. S. (1992). Seasonal cycles of growth, development and movement of the African honey bee, *Apis mellifera* scutettata, in Africa. *Insectes Sociaux* 39, 167–179. doi: 10.1007/BF01249292

Moore, D. (2016). Honey bee circadian clocks: behavioral control from individual workers to whole-colony rhythms. *J. Insect Physiol.?* 47, 843–857. doi: 10.1016/S0022-1910(01)00057-9

Moritz, B., and Crailsheim, K. (1987). Physiology of protein digestion in the midgut of the honeybee (*Apis mellifera* L.). *J. Insect Physiol.* 33, 923–931. doi: 10.1016/0022-1910(87)90004-7

Omholt, S. W. (1988). Relationships between worker longevity and the intracolonial population dynamics of the honeybee. *J. Theor. Biol.* 130, 275–284. doi: 10.1016/S0022-5193(88)80030-4

Pinto, M. A., Sheppard, W. S., Johnston, J. S., Rubink, W. L., Coulson, R. N., Schiff, N. M., et al. (2007). Honey bees (Hymenoptera: Apidae) of African origin exist in non-Africanized areas of the Southern United States: evidence from mitochondrial DNA. *Ann. Entomol. Soc. Am.* 100, 289–295.

Renzi, M. T., Rodríguez-Gasol, N., Medrzycki, P., Porrini, C., Martini, A., Burgio, G., et al. (2016). Combined effect of pollen quality and thiamethoxam on hypopharyngeal gland development and protein content in *Apis mellifera*. *Apidologie* 47, 779–788. doi: 10.1007/s13592-016-0435-9

Rivera-Marchand, B., Giray, T., and Guzman-Novoa, E. (2008). The cost of defense in social insects: insights from the honey bee. *Entomol. Exp. Appl.* 129, 1–10.

Rivera-Marchand, B., Oskay, D., and Giray, T. (2012). Gentle Africanized bees on an oceanic island. *Evol. Appl.* 5, 746–756. doi: 10.1111/j.1752-4571.2012.00252.x

Seeley, T. D. (1989). The honey bee colony as a superorganism. *Am. Sci.* 77, 546–553.

Seeley, T. D., and Visscher, P. K. (1985). Survival of honeybees in cold climates: the critical timing of colony growth and reproduction. *Ecol. Entomol.* 10, 81–88. doi: 10.1111/j.1365-2311.1985.tb00537.x

Shechtman, O. (2013). "The coefficient of variation as an index of measurement reliability," in *Methods of Clinical Epidemiology*, eds S. A. R. Doi and G. M. Williams (Berlin: Springer), 39–49. doi: 10.1007/978-3-642-37131-8_4

Smedal, B., Brynem, M., Kreibich, C. D., and Amdam, G. V. (2009). Brood pheromone suppresses physiology of extreme longevity in honeybees (*Apis mellifera*). *J. Exp. Biol.* 212(Pt 23), 3795–3801. doi: 10.1242/jeb.035063

Smodiš Škerl, M. I., and Gregorc, A. (2015). Characteristics of hypopharyngeal glands in honeybees (*Apis mellifera* carnica) from a nurse colony. *Slov. Vet. Res.* 52, 67–74.

Southwick, E. E. (1991). "Overwintering in honey bees: implications for apiculture," in *Insects at Low Temperature*, eds R. E. Lee and D. L. Denlinger (Boston, MA: Springer).

Stabentheiner, A., Pressl, H., Papst, T., Hrassnigg, N., and Crailsheim, K. (2003). Endothermic heat production in honeybee winter clusters. *J. Exp. Biol.* 206, 353–358. doi: 10.1242/jeb.00082

Vannette, R. L., Mohamed, A., and Johnson, B. R. (2015). Forager bees (*Apis mellifera*) highly express immune and detoxification genes in tissues associated with nectar processing. *Sci. Rep.* 5:1622. doi: 10.1038/srep16224

Villa, J. D., and Rinderer, T. E. (1993). Cold room thermoregulation, store consumption, and survival of Africanized and European honey bees (*Apis mellifera* L). *Apidologie* 24, 411–423. doi: 10.1051/apido:19930408

Winston, M. L., Taylor, O. R., Otis, G. W., Winston, M. L., Taylor, O. R., Otis, G. W., et al. (2016). Some differences between temperate european and tropical African and South American Honeybees. *Bee World* 64, 12–21. doi: 10.1080/0005772X.1983.11097902

Withrow, J. M., and Tarpy, D. R. (2018). Cryptic "royal" subfamilies in honey bee (*Apis mellifera*) colonies. *PLoS One* 13:e0199124. doi: 10.1371/journal.pone.0199124

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Neonicotinoid Pesticides Are More Toxic to Honey Bees at Lower Temperatures: Implications for Overwintering Bees

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The honey bee (*Apis mellifera*) is an important pollinator as well as an important test model for pesticide effects on other insect pollinators. Honey bees have been experiencing high mortality in both the United States and worldwide. Pesticide exposure has been identified as one of the many stressors causing this mortality. Effects of various pesticides have been measured for multiple responses such as learning, memory performance, feeding activity, and thermoregulation. These studies were conducted at many different temperatures (11–35°C); however, few studies compared toxicity of the same pesticide to bees at different temperatures. It is possible that the same pesticide might show different toxicity to honey bees at different temperatures. To reveal such potential interactions, we administered low doses of two neonicotinoid insecticides (imidacloprid and thiamethoxam) at three different temperature scenarios (35°C, 24°C, and a varying temperature) and determined the effects on honey bee survivorship. We discovered that honey bees are much more sensitive to the neonicotinoid pesticides imidacloprid and thiamethoxam at a constant 24°C or at a varying temperature (night at 13°C and day at 24°C) compared to bees at 35°C. These results suggest that honey bee colonies during winter time will be more sensitive to pesticides. Doses of neonicotinoids that are safe to colonies during summer might kill them during the winter time.

Keywords: overwintering bees, temperature, neonicotinoids, pesticide toxicity, *Apis mellifera*

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INTRODUCTION

Pollinators are responsible for the transfer of pollen between flowers, helping fruit and seed production in approximately 88% of flowering plants (Ollerton et al., 2011). Whereas a wide variety of animal taxa can work as pollinators (Ollerton, 2017), honey bees (*Apis mellifera*) are highly efficient in pollen transfer and are used for the majority of pollination services in both cultivated and wild plants (Willmer et al., 2017).

Pesticides are widely used in agriculture, but they can also be devastating to the health of many non-target organisms (Desneux et al., 2007; Schäfer et al., 2012). Recently, great attention has been paid to the effects of neonicotinoid pesticides on honey bees and their potential role in harming the health of honey bee colonies all over the world (Matsumoto, 2013). These systemic insecticides are strong agonists of the nicotinic acetylcholine receptors (nAChR), mainly circulated in the insect central nervous system, and can interrupt processes related to cholinergic neurotransmission, such as olfaction, learning, and memory (Jones et al., 2006; Williamson and Wright, 2013). Currently, 30% of the insecticides used worldwide are neonicotinoids (Simon-Delso et al., 2015), and honey bees are exposed to neonicotinoids both in the field and inside the hive (Lambert et al., 2013). The sublethal effects of neonicotinoids on honey bees have been extensively studied at many different physiological levels (Aliouane et al., 2009; Henry et al., 2012; Catae et al., 2014; Oliveira et al., 2014; Alburaki et al., 2015), but whether this toxicity interacts with low temperature has not yet been explored.

Honey bees are distributed in a wide geographic range, with greatly differing climatic conditions (Ruttner, 1988). This wide range includes many areas with cold and challenging winters. Successful overwintering of honey bee colonies is crucial to meet the pollination requirements of early spring blossoming crops like as cherries, apples, and almonds (Doeke et al., 2015).

Honey bees survive cold winters through the development of a special type of bees called "winter bees" (Maurizio, 1950; Münch and Amdam, 2010). Winter bees have vital endocrine and metabolic changes that differ from summer adult worker honey bees, which may increase their lifespan by 6–8 times (Huang and Wang, 2015). Numerous studies have found that winter bees have low levels of juvenile hormone as compared to summer bees and high levels of vitellogenin and total proteins in the hemolymph (Fluri et al., 1982; Huang and Robinson, 1995; Hartfelder and Engels, 1998); reduced protein synthesis, transportation through the midgut, and catabolism (Crailsheim, 1990; Haszonits and Crailsheim, 1990); and lesser activity of monooxygenase enzymes that are essential for detoxification of pesticides (Smirle and Winston, 1987). Yet, it remains ambiguous how the physiological variations between summer and winter workers change pesticide sensitivity. One study showed that winter bees are less sensitive to a fungicide (imidazole) and an insecticide (pyrethroid) (Meled et al., 1998), but another one showed higher sensitivity in winter bees (in the spring, after winter was over) to thiamethoxam and clothianidin compared to summer workers (Baines et al., 2017). It is possible that near the end of the winter, these bees might be more similar to summer foragers after their fat and vitellogenin levels are depleted due to brood rearing.

Neonicotinoids have been widely studied for direct effects on honey bees (Gill et al., 2012) as well as changes in behaviors, such as learning (Decourtye et al., 2003; El Hassani et al., 2008; Aliouane et al., 2009), memory performance (Alix et al., 2009; Thompson, 2010), and feeding activity (Blacquière et al., 2012). Effects of thiamethoxam and clothianidin have been shown to affect survival at 29°C (Wood et al., 2020) and thermoregulation at 22 and 33°C (Tosi et al., 2016); however, almost no studies compared the toxicity of the same pesticide to bees at different temperatures. Brood-nest temperature is usually regulated at close to 35°C (Li et al., 2016) but many pesticide toxicities are conducted at room temperature (25°C) (e.g., Burley et al., 2008; Lushchak et al., 2018; and recommended as a standard method by Medrzycki et al., 2013). It is possible that the same pesticide might show different toxicity to honey bees at different temperatures but this is not studies for neonicotinoid pesticides.

Honey bees can be exposed to pesticides via either nectar/honey (energy source), or via pollen (protein source). Honey samples have been shown to contain high enough levels of neonicotinoids to affect honey bees (Mitchell et al., 2017), with the average total concentration of the five measured neonicotinoids at 1.8 ng/g in contaminated samples and a maximum of 56 ng/g. Pollen samples are even worse: almost 60% of samples contained at least one pesticide, with imidacloprid as high as 206 ppb (Mullin et al., 2010). Because an individual honey bee needs to consume about 11 mg of sugar (using honey) per day (Huang et al., 1998) and bees will also consume pollen before becoming winter bees (Maurizio, 1950), bees during winter will also be exposed to pesticides through both of these pathways.

In this study, we tested the effects of sublethal doses of the neonicotinoids thiamethoxam and imidacloprid, two commonly used insecticides at different temperatures, representing different conditions experienced by bees: constant 24 (room temperature), constant 35°C (brood-nest temperature), and 13°C/24°C (representing common night and day time temperatures). The objective of this study was to evaluate whether two neonicotinoid pesticides show different toxicity to honey bees at different temperatures. To meet this objective, we performed multiple cage trials. Firstly, we examined the survival of honey bees exposed to imidacloprid and thiamethoxam at 24°C compared to 35°C, using bees from the brood-nest (Trial 1) and using older workers from outside the cluster (Trial 2). Next, we compared the survival effects of exposure to these two pesticides for older workers at 35°C compared to a constant low temperature (23) and a normal winter temperature variation (ranging from 13°C to 24°C, Trial 3).

MATERIALS AND METHODS

Three cage trials were conducted in November 2019 at the Honey Bee Biology Lab, Michigan State University, United States (with colonies at 42°40′45.1″N 84°28′38.8″W). In Trial 1, bees from the brood-nest were used. In Trials 2 and 3, older workers near the top of the winter cluster were sampled with an insect vacuum and then brought into the lab. All bees were anaesthetized with carbon dioxide and divided into cages. The wooden cages (14 × 12 × 9 cm) had mesh in the bottom, a piece transparent plastic sheet with holes in the front, two holes in the top with space for inserting feeding vials, and a piece of cloth was fixed at the center of each cage for possible clustering, if needed. After collection of bees, prior to the start of each trial, the bees were given a 24 h acclimatization period, during which they received untreated 50% sucrose solution. Bees that died during the acclimatization period were removed and excluded from analysis.

In Trial 1 six cages with 40 young bees each were monitored, and Trial 2 six cages containing 25 to 35 older bees, were monitored (**Supplementary Table S1**). In each trial, three cages were kept in an incubator set at 35°C (brood-nest temperature) and three cages were kept in an incubator set at 24°C (room temperature). Both incubators were maintained at 50% relative humidity. At the end of both Trials 1 and 2 (end of 4 days),

TABLE 1 | Recorded temperatures of one typical day (day 5) of the three trials.

Trial (Colony ID)	Planned temperature	Actual temperature (°C) (mean ± SE)
1 (A)	Brood-nest	34.75 ± 0.23
	Low	23.76 ± 0.02
2 (B)	Brood-nest	34.80 ± 0.03
	Low	23.27 ± 0.10
3 (C, D, E)	Brood-nest	34.43 ± 0.09
	Low	23.28 ± 0.72
	Varying	Day: 24.19 ± 0.13 Night: 13.22 ± 0.01

N = 48 (every 30 min) for all data points except day and night (N = 24).

the bees that were kept at 35°C were transferred to the 24°C incubator, and all bees were monitored for another day.

In Trial 3, 25 to 40 bees per cage were housed at three temperatures (**Supplementary Table S1**), with 9 cages (3 treatments × 3 colonies) for each of the following three different temperatures: 35°C, 13 to 24°C, and room temperature (23°C, insider a drawer in complete darkness). The 13–24°C treatment was a changing temperature setting with the incubator programmed to have the temperature at 24°C during the day (7:00 am to 6:00 pm) and 13°C at night (6:30 pm to 6:30 am) to simulate the natural temperature variation during late fall or early winter. Each temperature had three cages of bees (two with pesticides and one control).

Bees were provided with 0.4 ng/μl (0.25 ppm) imidacloprid, or 0.2 ng/μl (0.125 ppm) thiamethoxam (dissolved in acetone then added to syrup) or no pesticide (control). Control bees received the same amount of acetone (12.5 μl in 20 ml of sugar syrup). In Trial 2, bees received a half dose of the pesticides for the first day, i.e., 0.2 ng/μl (0.125 ppm) imidacloprid or 0.1 ng/μl (0.0625 ppm) thiamethoxam. After the first day we adjusted to the same dose as Trials 1 and 3 for the remainder of the experiment.

Statistical Analysis

Data in three trials were analyzed using R 3.5.2 using Kaplan–Meier survival analysis and Log Rank Test test. For Trial 3, three-way ANOVA was also used to analyze the mortality differences among the three temperatures, main treatment (two pesticides and one control) and the three colonies. Mortality data were first transformed [arcsine (square root of mortality)] to make the data fit a normal distribution. Standard errors are reported after the mean.

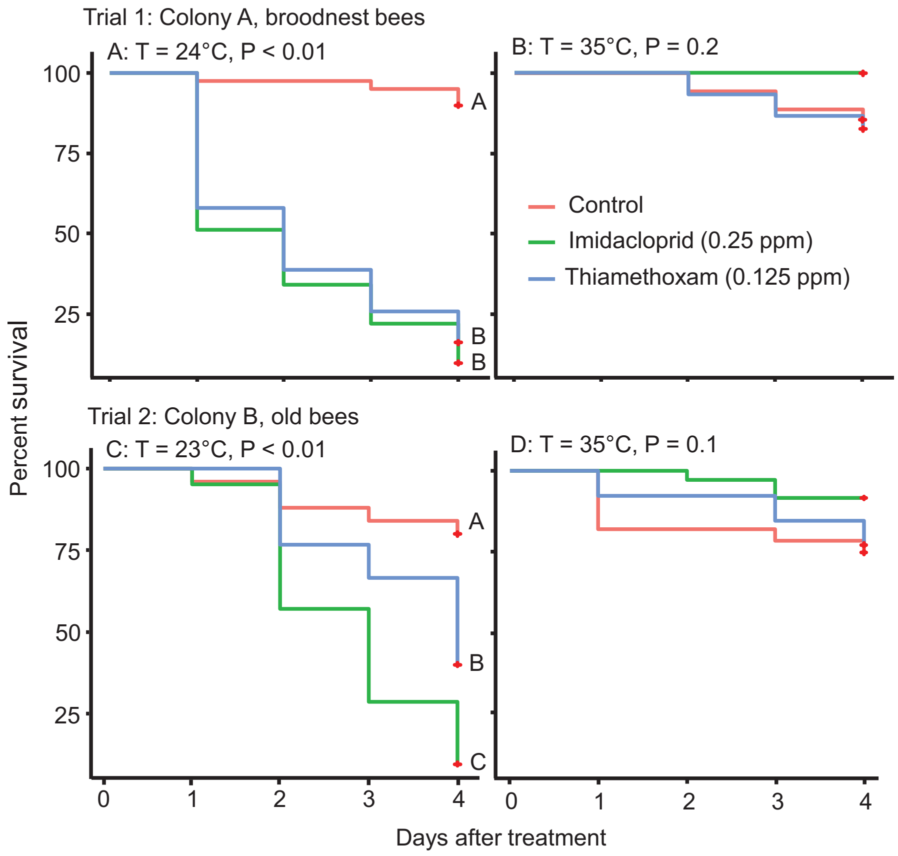

FIGURE 1 | Results of Trials 1 and 2 showing Kaplan–Meier survival curves of bees fed with imidacloprid or thiamethoxam or sugar only (control) at 23/24 at 24°C (**left**, **A**,**C**) or 35°C (**right**, **B**,**D**). Different letters at each line indicate significant differences with Log Rank tests at *P* < 0.05.

RESULTS

Digital recordings of temperatures are presented in **Table 1** during each of the three trials.

There was a significant negative effect of both neonicotinoid insecticides on the survival of caged honey bees compared to the control when kept at 24°C (Log Rank Test; $X^2 = 63.5$, df = 2, $P < 0.001$, **Figure 1A**, Trial 1). This negative effect on survival was not seen when bees were kept at 35°C (Log Rank Test; $X^2 = 3.4$, df = 2, $P = 0.2$, **Figure 1B**).

Similar effects negative effects on survival were seen in older workers (Trial 2). When the survival data were analyzed over the 4 days, there was a significant negative effect of both neonicotinoid insecticides on the survival of caged honey bees at 23°C; the survival of bees between the two pesticides was also significantly different, with imidacloprid having a greater negative effect on survival (Log Rank Test; $X^2 = 24.3$, df = 2, $P < 0.001$, **Figure 1C**). In contrast, there was no significant difference in bee survival between the bees fed with either pesticide and the control when bees were kept at 35°C (Log Rank Test; $X^2 = 3.8$, df = 2, $P = 0.1$, **Figure 1D**). We explored a lower dose (half compared to Trial 1) in Trial 2 for the first day, but no mortality was observed so we adjusted to the same doses as Trial 1 on the second day and observed bees for another 4 days.

At the end of the 4-day survival trials (Trials 1 and 2), we transferred the caged bees (and their food) that were at 35°C to 24°C to determine if pesticides lost their effectiveness at the higher temperature, explaining the differential mortality in bees. In both trials, mortality of bees of a single day after temperature change (from 35 to 24°C, due to the transfer) was significantly higher in the pesticide-treated bees (paired *t*-test, $P < 0.05$), but not for the control bees (**Table 2**).

In Trial 3, survival of bees under low temperature (23°C) was similar to bees under varying temperature (13°C–24°C), showing a strong negative effect of pesticides on survival (Log Rank Test; $X^2 = 34.8$, df = 2, $P < 0.001$ for 13–24°C; Log Rank Test; $X^2 = 136$, df = 2, $P < 0.001$ for 23°C). Bee survival at brood-nest temperature (35°C) was much higher with only one pesticide (imidacloprid) showing a slight, but significant effect (Log Rank Test; $X^2 = 10.3$, df = 2, $P = 0.006$ for 23°C, **Figure 2**).

TABLE 2 | Mortality (%) of bees before and after being transferred from 35°C to 24°C to determine whether pesticides were still active.

	Mortality at 35°C (4 days)	Mortality at 24°C (1 day)
Trial 1		
Control	7.7	16.2
Imidacloprid	0.0	52.0
Thiamethoxam	15.0	41.1
Trial 2		
Control	23.3	21.7
Imidacloprid	10.0	92.3
Thiamethoxam	25.7	92.3

Paired t-test (not including control bees) showed a significant difference between the two temperatures (P = 0.018), despite of the fact that the mortality of 35°C was during 4 days and those at 24°C were for 1 day only.

The total mortality over the 5 days were transformed and analyzed by a three-way ANOVA. There were no significant differences among the three colonies [$F(2, 18) = 0.61$, $P > 0.5$] in mortality so further analysis did not include colony in the model. There were significant differences among the different temperatures [$F(2, 18) = 121.1$, $P < 0.01$], treatment (pesticides or control) [$F(2, 18) = 44.6$, $P < 0.01$], and also significant interactions between the temperature and treatment [$F(4, 18) = 11.0$, $P < 0.01$]. **Figure 3** shows the changes of mortality among the three pesticide treatments at three different temperatures.

DISCUSSION

To our understanding, no studies have been carried out on the toxicity of neonicotinoids to winter bees at different temperatures with the same doses. In our study, the major findings are that the imidacloprid (0.25 ppm) and thiamethoxam (0.125 ppm) are more harmful to *Apis mellifera* at a lower temperature (25°C, normally considered as "room temperature" in many pesticide tests) compared to those at the brood-nest temperature (35°C). We clearly show that temperature should be considered when evaluating risk of pesticides to honey bees.

Honey bee declines have multiple causes and may include parasites, pathogens, pesticides, nutrition/habitat loss/climate change, and transportation (Goulson et al., 2015). Recent attention has focused on how neonicotinoids affect honey bees. Baines et al. (2017) revealed significant negative effects of environmental concentrations of thiamethoxam and clothianidin on winter adult worker bees' survival under laboratory conditions. More recently, another study reported chronic exposure of thiamethoxam (0.0049, 0.0195, 0.0973 ppm) and clothianidin (0.0042, 0.0167, 0.0832 ppm) significantly reduced survival of winter workers as compared to the control at all tested doses under laboratory conditions (Wood et al., 2020). However, these studies used one single temperature (29°C) for testing. Honey bees are ectotherms when brood is absent, and their body temperature and many of their biological functions are dependent on the environmental temperature. Bees in the center of broodless winter cluster experience a broad range of temperatures (12–33.5°C), with bees at the periphery of the cluster reaching 6°C (Fahrenholz et al., 1989). Numerous studies have documented that the response to pesticides is temperature dependent (Lushchak et al., 2018). Honey bees show negative temperature coefficients in DDT (Ladas, 1972) and cabamates (Georghiou and Atkins, 1964). Our data show similar negative temperature coefficients for both imidacloprid and thiamethoxam. However, we did not directly compare the same breed of honey bees in summer and in winter. It is not clear if they will show similar sensitivity but we postulate that winter bees should be less sensitive because of their higher levels of vitellogenin which reduces oxidative stress (Seehuus et al., 2006), and winter bees already have consumed much pollen which also increases their stress resistance (Huang, 2012). We failed to find a difference though between Trial 1 (brood-nest

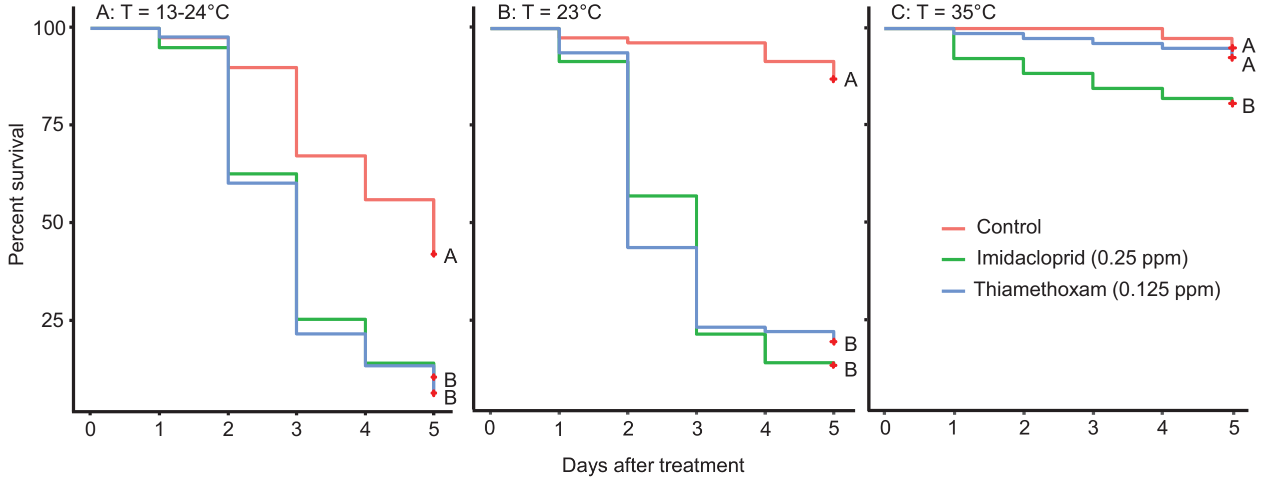

FIGURE 2 | Results of Trial 3 showing Kaplan–Meier survival curves of bees fed with imidacloprid or thiamethoxam or sugar only (control) at 13–24°C (**left**, **A**), 23°C (**middle**, **B**), or 35°C (**right**, **C**). Different letters at each line indicate significant differences with Log Rank tests at $P < 0.05$.

bees, more likely to be winter bees) and Trials 2 and 3, with old bees outside the cluster (more likely have already foraged in the fall and not real winter bees). Future studies should have better control on these aspects, e.g., with offspring from the same queens, some tested during summer, and some tested as marked real winter bees (e.g., those emerged in November in Michigan, United States).

We found that only a slight decrease (about 11°C) in temperature significantly increased toxicity of both pesticides to bees in all three trials. However, raising bees in a variable temperature environment (day 24°C, night 13°C) did not increase mortality significantly compared to a static cold temperature (23°C). We originally hypothesized that the variable temperature setting should be even more stressful to honey bees, because with presumably higher metabolic demands at 13°C at night, bees might become even more sensitive to pesticides. Instead, we did observe a significant increase of control mortality at the variable temperature (63% ±0.04) compared to 19.2% ±0.06 at 23°C, and 6.7% ±0.03 at 35°C, but no significant difference between the low and variable temperatures, for both pesticides (**Figure 2**). It is possible that even at 25°C, the mortality was already too high (92–93%) so no further increase was possible. But the survival curves of the three different temperatures (**Figure 2**) showed similar mortality for 23°C and 13–24°C at days 2–4. These results suggest that a variable temperature at day and night does not significantly change honey bee sensitivity to pesticides compared to a constant low temperature, but a reduction from the brood-nest temperature does make honey bees more sensitive to pesticides. This is rather intriguing because honey bee will forage fine at lower temperatures; in fact, *A. mellifera* has a preferred foraging temperature of 20°C (Tan et al., 2012) or 23°C (Verma and Dulta, 1986).

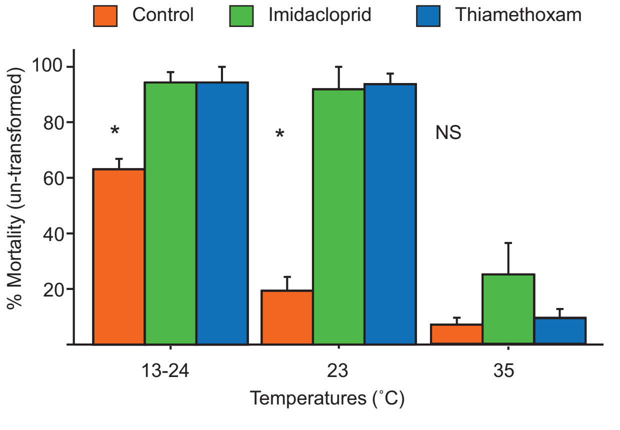

FIGURE 3 | Mean mortality (+SE) of bees exposed to the two pesticides or sugar only (control) at a varying (13°C at night and 24°C during day), constant low (23°C), or brood-nest (35°C) temperature. Control bees showed the highest, intermediate, and lowest mortality, respectively, at 13–24°C, 23°C, and 35°C (all pairwise comparisons significant, *t*-test, $P < 0.05$). The two pesticides showed similar high mortality at the two lower temperatures (different from the control, $P < 0.01$); but at 35°C, there was no significantly difference in mortality among the three treatments ($P > 0.05$). Statistics done on transformed data but figure here presented un-transformed data. * Denotes significant difference ($P < 0.05$) between the control and the two pesticides with Fisher's Protected Least Significant Difference test.

One possibility of our observed low toxicity at 35°C for both pesticides is that the pesticides were degraded right away at this high temperature. However, this is not the case because when we transferred bees at 35°C to 24°C on day 5, the caged bees again showed high mortality (**Table 2**), suggesting that the pesticides were still present in sugar syrup. The low mortality of pesticide-fed bees at 35°C, therefore, must be due to higher tolerance of bees at that temperature.

The one day that the bees received a smaller pesticide dose (day 1, Trial 2) suggests that the differential sensitivity of bees at different temperatures may be dose-dependent. During the day that the bees received a lower dose, we did not see mortality as we did in Trial 1. The dose chosen for the trial was calculated based on many studies (Decourtye et al., 2004; Yang et al., 2008; Eiri and Nieh, 2012; Henry et al., 2012; Schneider et al., 2012; Teeters et al., 2012; Derecka et al., 2013; Sandrock et al., 2014; Tan et al., 2014; and Williamson et al., 2014). Because so little is known about neonicotinoid exposures to winter bees in a diversity of contexts, it will be important to evaluate these findings over a broad range of doses and exposure scenarios. Further study is needed to identify the effects of lower doses at a variety of temperatures, as well as other realistic exposure scenarios including mixtures of pesticides.

CONCLUSION

We conclude that bees are more sensitive to neonicotinoids at lower temperatures, but a varying temperature does not exacerbate this sensitivity. Honey bee colonies are maintained at 35°C when there is brood, but during the broodless winter period honey bee colonies no longer regulate their temperature to 35°C (Jones et al., 2005). A dose that does not kill honey bees might suddenly cause colony loss during the wintering period. The higher resistance of bees at the broodnest temperature to neonicotinoid pesticide is intriguing, but perhaps not surprising: workers might be more adapted at this temperature compared to lower temperatures because it is more commonly experienced. For example, it is possible that their detoxifying enzymes show the highest activity at this optimal temperature. Further experiments are needed to study the mechanisms of this temperature-dependent pesticide sensitivity in honey bees.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

ZYH conceived and designed the experiments, collected the bees, and analyzed the data. MSS conducted the experiments. MOM contributed the materials. All authors contributed to the writing and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2020.556856/full#supplementary-material

REFERENCES

Alburaki, M., Boutin, S., Mercier, P. L., Loublier, Y., Chagnon, M., and Derome, N. (2015). Neonicotinoid-coated *Zea mays* seeds indirectly affect honeybee performance and pathogen susceptibility in field trials. *PLoS One* 10:e0125790. doi: 10.1371/journal.pone.0125790

Aliouane, Y., Adessalam, K., El Hassani, A. K., Gary, V., Armengaud, C., Lambin, M., et al. (2009). Subchronic exposure of honeybees to sublethal doses of pesticides: effect on behavior. *Environ. Toxicol. Chem.* 28, 113–122. doi: 10.1897/08-110.1

Alix, A., Chauzat, M. P., Duchard, S., Lewis, G., Maus, C., Miles, M. J., et al. (2009). Environmental risk assessment scheme for plant protection products. *Chapter* 10: Honeybees proposed scheme. *Julius-Kühn Arch.* 423, 27–33.

Baines, D., Wilton, E., Pawluk, A., deGorter, M., and Chomistek, N. (2017). Neonicotinoids act like endocrine disrupting chemicals in newly-emerged bees and winter bees. *Sci. Rep.* 7:10979.

Blacquière, T., Smagghe, G., van Gestel, C. A. M., and Mommaerts, V. (2012). Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment. *Ecotoxicology* 21, 973–992. doi: 10.1007/s10646-012-0863-x

Burley, L. M., Fell, R. D., and Saacke, R. G. (2008). Survival of honey bee (Hymenoptera: Apidae) spermatozoa incubated at room temperature from drones exposed to miticides. *J. Econ. Entomol.* 101, 1081–1087. doi: 10.1603/0022-0493(2008)101[1081:sohbha]2.0.co;2

Catae, A. F., Roat, T. C., De Oliveira, R. A., Nocelli, R. C. F., and Malaspina, O. (2014). Cytotoxic effects of thiamethoxam in the midgut and malpighian tubules of Africanized *Apis mellifera* (Hymenoptera: Apidae). *Microsc. Res. Tech.* 77, 274–281. doi: 10.1002/jemt.22339

Crailsheim, K. (1990). The protein balance of the honey bee worker. *Apidologie* 21, 417–429. doi: 10.1051/apido:19900504

Decourtye, A., Devillers, J., Cluzeau, S., Charreton, M., and Pham-Delègue, M. H. (2004). Effects of imidacloprid and deltamethrin on associative learning in honeybees under semi-field and laboratory conditions. *Ecotoxicol. Environ. Saf.* 57, 410–419. doi: 10.1016/j.ecoenv.2003.08.001

Decourtye, A., Lacassie, E., and Pham-Delegue, M. H. (2003). Learning performances of honeybees (*Apis mellifera L.*) are differentially affected by imidacloprid according to the season. *Pest Manag Sci.* 59, 269–278. doi: 10.1002/ps.631

Derecka, K., Blythe, M. J., Malla, S., Genereux, D. P., Guffanti, A., and Pavan, P. (2013). Transient exposure to low levels of insecticide affects metabolic networks of honeybee larvae. *PLoS One* 8:e68191. doi: 10.1371/journal.pone.0068191

Desneux, N., Decourtye, A., and Delpuech, J. M. M. (2007). The sublethal effects of pesticides on beneficial arthropods. *Annu. Rev. Entomol.* 52, 81–106. doi: 10.1146/annurev.ento.52.110405.091440

Doeke, M. A., Frazier, M., and Grozinger, C. M. (2015). Overwintering honey bees: biology and management. *Curr. Opin. Insect Sci.* 10, 185–193. doi: 10.1016/j.cois.2015.05.014

Eiri, D. M., and Nieh, J. C. (2012). A nicotinic acetylcholine receptor agonist affects honey bee sucrose responsiveness and decreases waggle dancing. *J. Exp. Biol.* 215(Pt. 12), 2022–2029. doi: 10.1242/jeb.068718

El Hassani, A. K., Dacher, M., Garry, V., Lambin, M., Gauthier, M., and Armengaud, C. (2008). Effects of sublethal doses of acetamiprid and thiamethoxam on the behavior of the honeybee (*Apis mellifera*). *Arch. Environ. Contam. Toxicol.* 54, 653–661. doi: 10.1007/s00244-007-9071-8

Fahrenholz, L., Lamprecht, I., and Schricker, B. (1989). Thermal investigations of a honey bee colony: thermoregulation of the hive during summer and winter and heat production of members of different bee castes. *J. Comp. Physiol. B* 159, 551–560. doi: 10.1007/bf00694379

Fluri, P., Lüscher, M., Wille, H., and Gerig, L. (1982). Changes in weight of the pharyngeal gland and haemolymph titres of juvenile hormone, protein and vitellogenin in worker honey bees. *J. Insect Physiol.* 28, 61–68. doi: 10.1016/0022-1910(82)90023-3

Georghiou, G. P., and Atkins, Jr, E. L (1964). Temperature coefficient of toxicity of certain n-methylcarbamates against honeybees, and the effect of the synergist piperonyl butoxide. *J. Apicult. Res.* 3, 31–35. doi: 10.1080/00218839.1964.11100079

Gill, R. J., Ramos-Rodriguez, O., and Raine, N. E. (2012). Combined pesticide exposure severely affects individual- and colony-level traits in bees. *Nature* 491, 105–108. doi: 10.1038/nature11585

Goulson, D., Nicholls, E., Botías, C., and Rotheray, E. L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science.* 347:1255957. doi: 10.1126/science.1255957

Graves, J. B., and Mackensen, O. (1965). Topical application and insecticide resistance studies on the honey bee. *J. Econ. Entomol.* 58, 990–993. doi: 10.1093/jee/58.5.990

Hartfelder, K., and Engels, W. (1998). Social insect polymorphism: Hormonal regulation of plasticity in development and reproduction in the honeybee. *Curr. Top. Dev. Biol.* 40, 45–77. doi: 10.1016/s0070-2153(08)60364-6

Haszonits, O., and Crailsheim, K. (1990). Uptake of L-leucine into isolated enterocytes of the honeybee (*Apis mellifera L.) depending on season. J. Insect Physiol.* 36, 835–842. doi: 10.1016/0022-1910(90)90170-k

Henry, M., Rollin, O., Aptel, J., Tchamitchian, S., Beguin, M., Requier, F., et al. (2012). A common pesticide decreases foraging success and survival in honey bees. *Science*336, 348–350. doi: 10.1126/science.1215039

Huang, Z. Y. (2012). Pollen nutrition affects honey bee stress resistance. *Terrest. Arth. Rev.* 5, 175–189. doi: 10.1163/187498312x639568

Huang, Z.-Y., Plettner, E., and Robinson, G. E. (1998). Effect of social environment and mandibular gland removal on division of labor in worker honey bees. *J. Comp. Physiol. B* 183, 143–152.

Huang, Z. Y., and Robinson, G. E. (1995). Seasonal changes in juvenile hormone titers and rates of biosynthesis in honey bees. *J. Comp. Physiol.* 165, 18–28. doi: 10.1007/bf00264682

Huang, Z. Y., and Wang, Y. (2015). "Social physiology of honey bees: differentiation in behaviors, castes, and longevity," in *The Hive and the Honey Bee*, ed. J. Graham (Hamilton, IL: Dadant and Sons Press), 183–200.

Jones, A. K., Raymond-Delpech, V., Thany, S. H., Gauthier, M., and Sattelle, D. B. (2006). The nicotinic acetylcholine receptor gene family of the honey bee, *Apis mellifera*. *Genome Res.* 16, 1422–1430. doi: 10.1101/gr.4549206

Jones, J. C., Helliwell, P., Beekman, M., Maleszka, R., and Oldroyd, B. P. (2005). The effects of rearing temperature on developmental stability and learning and memory in the honey bee, *Apis mellifera*. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* 191, 1121–1129. doi: 10.1007/s00359-005-0035-z

Ladas, A. (1972). The influence of some internal and external factors upon the insecticide resistance of honeybee. *Apidologie* 3, 55–78.

Lambert, O., Piroux, M., Puyo, S., Thorin, C., L'Hostis, M., Wiest, L., et al. (2013). Widespread occurrence of chemical residues in beehive matrices from apiaries located in different landscapes of western France. *PLoS One* 8:e67007. doi: 10.1371/journal.pone.0067007

Li, Z. Y., Huang, Z. Y., Sharma, D. B., Xue, Y., Wang, Z., and Ren, B. (2016). Drone and worker brood microclimates are regulated deferentially in honey bees, *Apis mellifera*. *PLoS One* 11:e0148740. doi: 10.1371/journal.pone.0148740

Lushchak, V. I., Matviishyn, T. M., Husak, V. V., Storey, J. M., and Storey, K. B. (2018). Pesticide toxicity: a mechanistic approach. *EXCLI J.* 17, 1101–1136. doi: 10.17179/excli2018-1710

Matsumoto, T. (2013). Reduction in homing flights in the honey bee Apis Mellifera after a sublethal dose of neonicotinoid insectcides. *Bull. Insectol.* 66, 1–9.

Maurizio, A. (1950). The influence of pollen feeding and brood rearing on the length of life and physiological conditions of the honeybee. *Bee World.* 1950, 9–12. doi: 10.1080/0005772X.1950.11094617

Medrzycki, P., Giffard, H., Aupinel, P., Belzunces, L. P., Chauzat, M. P., Claßen, C., et al. (2013). Standard methods for toxicology research in *Apis mellifera*. *J. Apicult. Res.* 52, 1–60. doi: 10.3896/ibra.1.52.4.23

Meled, M., Thrasyvoulou, A., and Belzunces, L. P. (1998). Seasonal variations in susceptibility of *Apis mellifera* to the synergistic action of prochloraz and deltamethrin. *Environ. Toxicol. Chem.* 17, 2517–2520. doi: 10.1897/1551-5028(1998)017<2517:svisoa>2.3.co;2

Mitchell, E. A., Mulhauser, B., Mulot, M., Mutabazi, A., Glauser, G., and Aebi, A. (2017). A worldwide survey of neonicotinoids in honey. *Science* 358, 109–111. doi: 10.1126/science.aan3684

Mullin, C. A., Frazier, M., Frazier, J. L., Ashcraft, S., Simonds, R., vanEngelsdorp, D., et al. (2010). High levels of miticides and agrochemicals in north american apiaries: implications for honey bee health. *PLoS One* 5:e9754. doi: 10.1371/journal.pone.0009754

Münch, D., and Amdam, G. V. (2010). The curious case of aging plasticity in honey bees. *FEBS Lett.* 584, 2496–2503. doi: 10.1016/j.febslet.2010.04.007

Oliveira, R. A., Roat, T. C., Carvalho, S. M., and Malaspina, O. (2014). Side-effects of thiamethoxam on the brain and midgut of the Africanized honeybee *Apis mellifera* (Hymenpptera: Apidae). *Environ. Toxicol.* 29, 1122–1133. doi: 10.1002/tox.21842

Ollerton, J. (2017). Pollinator diversity: distribution, ecological function, and conservation. *Annu. Rev. Ecol. Evol. Syst.* 48, 353–376. doi: 10.1146/annurev-ecolsys-110316-022919

Ollerton, J., Winfree, R., and Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos* 120, 321–326. doi: 10.1111/j.1600-0706.2010.18644.x

Ruttner, F. (1988). *Biogeography and Taxonomy of Honeybees*. Berlin: Springer-Verlag.

Sandrock, C., Tanadini, L. G., Pettis, J. S., Biesmeijer, J. C., Potts, S. G., and Neumann, P. (2014). Sublethal neonicotinoid insecticide exposure reduces solitary bee reproductive success. *Agric. Forest Entomol.* 16, 119–128. doi: 10.1111/afe.12041

Schäfer, R. B., von, der Ohe, P. C., Rasmussen, J., Kefford, B. J., Beketov, M. A., et al. (2012). Thresholds for the effects of pesticides on invertebrate communities and leaf breakdown in stream ecosystems. *Environ. Sci. Technol.* 46, 5134–5142. doi: 10.1021/es2039882

Schneider, C. W., Tautz, J., Grünewald, B., and Fuchs, S. (2012). RFID tracking of sublethal effects of two neonicotinoid insecticides on the foraging behavior of *Apis mellifera*. *PLoS One* 7:e30023. doi: 10.1371/journal.pone.0030023

Seehuus, S. C., Norberg, K., Gimsa, U., Krekling, T., and Amdam, G. V. (2006). Reproductive protein protects functionally sterile honey bee workers from oxidative stress. *Proc. Natl. Acad. Sci. U.S.A.* 103, 962–967. doi: 10.1073/pnas.0502681103

Simon-Delso, N., Amaral-Rogers, V., Belzunces, L. P., Bonmatin, J. M., Chagnon, M., Downs, C., et al. (2015). Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. *Environ. Sci. Pollut. Res.* 22, 5–34. doi: 10.1007/s11356-014-3470-y

Smirle, M. J., and Winston, M. L. (1987). Inter colony variation in pesticide detoxification by the honey bee (Hymenoptera: Apidae). *J. Econ. Entomol.* 80, 5–8. doi: 10.1093/jee/80.1.5

Tan, K., Chen, W., Dong, S., Liu, X., Wang, Y., and Nieh, J. C. (2014). Imidacloprid alters foraging and decreases bee avoidance of predators. *PLoS One* 9:e102725. doi: 10.1371/journal.pone.0102725

Tan, K., Yang, S., Wang, Z. W., Radloff, S. E., and Oldroyd, B. P. (2012). Differences in foraging and brood-nest temperature in the honey bees *Apis cerana* and *A. mellifera*. *Apidologie* 43, 618–623. doi: 10.1007/s13592-012-0136-y

Teeters, B. S., Johnson, R. M., Ellis, M. D., and Siegfried, B. D. (2012). Using video-tracking to assess sublethal effects of pesticides on honey Bees (*Apis mellifera* L.). *Environ. Toxicol. Chem.* 31, 1349–1354. doi: 10.1002/etc.1830

Thompson, H. M. (2010). Risk assessment for honey bees and pesticides: recent developments and 'new issues'. *Pest Manag. Sci.* 66, 1157–1162. doi: 10.1002/ps.1994

Tosi, S., Démares, F. J., Nicolson, S. W., Medrzycki, P., Pirk, C. W., and Human, H. (2016). Effects of a neonicotinoid pesticide on thermoregulation of African honey bees (*Apis mellifera scutellata). J. Insect Physiol.* 93, 56–63. doi: 10.1016/j.jinsphys.2016.08.010

Verma, L. R., and Dulta, P. C. (1986). Foraging behaviour of *Apis cerana indica* and *Apis mellifera* in pollinating apple flowers. *J. Apicult. Res.* 25, 197–201.

Williamson, S. M., Willis, S. J., and Wright, G. A. (2014). Exposure to neonicotinoids influences the motor function of adult worker honeybees. *Ecotoxicology* 23, 1409–1418. doi: 10.1007/s10646-014-1283-x

Williamson, S. M., and Wright, G. A. (2013). Exposure to multiple cholinergic pesticides impairs olfactory learning and memory in honeybees. *J. Exp. Biol.* 216, 1799–1807. doi: 10.1242/jeb.083931

Willmer, P. G., Cunnold, H., and Ballantyne, G. (2017). Insights from measuring pollen deposition: quantifying the pre-eminence of bees as flower visitors and effective pollinators. *Arthropod Plant Interact* 11, 411-425.

Wood, S. C., Kozii, I. V., Medici, de Mattos, I., de Carvalho Macedo, Silva, R., et al. (2020). Chronic high-neonicotinoid exposure decreases overwinter survival of *Apis mellifera* L. *Insects* 11:30. doi: 10.3390/insects11010030

Yang, E. C., Chuang, Y. C., Chen, Y. L., and Chang, L. H. (2008). Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). *J. Econ. Entomol.* 101, 1743–1748.

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Grooming Behavior in Naturally *Varroa*-Resistant *Apis mellifera* Colonies From North-Central Argentina

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The Western honey bee, *Apis mellifera,* is an important species in providing honey and pollination services globally. The mite *Varroa destructor* is the major threat to *A. mellifera,* and it is associated with the severe colony winter mortality reported in recent decades. However, *Varroa* mite tolerant or resistant populations of *A. mellifera* have been detected around the world. A proposed mechanism responsible for limiting mite population growth in the colonies is grooming behavior, the physical removal and injury of mites from the adult bee bodies by individual workers or by their nest-mates. This behavioral strategy has been poorly studied in *V. destructor*-resistant colonies worldwide, especially in honey bee populations of European origin. In Argentina, honey bee stocks showing survival without mite treatment have been reported. In the present study, European-derived *A. mellifera* populations established in the Transition Chaco eco-region (Santa Fe province), with a subtropical climate, were characterized at the colony level. A honey bee stock showing natural *Varroa*-resistance (M) was compared to a *Varroa*-susceptible stock (C) for parameters of colony status (colony strength, percentage of *Varroa* infestation in adults and brood, hygienic behavior) and for indirect measures of grooming (percentage of fallen mites and damaged mites). M colonies showed lower phoretic and brood infestation and higher hygienic behavior in early autumn, and higher survival and population strength after wintering, in comparison with C colonies. The mean percentages of fallen mites and of damaged mites, and the injury to mites were higher in M than in C colonies. Our results suggest that, by modulating the parasitization dynamics in colonies, grooming behavior would be associated with the higher survival of *Varroa*-resistant stock. This study sheds light on how honey bee colonies can adaptively respond to mite pressure by modeling their behavior to resist Varroosis and provides evidence for grooming as an emerging factor evolving by natural selection. Percentage of damaged mites appears to be a reliable measure to enhance this behavior in honey bee colonies by selective breeding. Finally, the importance of improving and protecting locally adapted honey bee populations with natural *Varroa* resistance for regional apiculture is discussed.

Keywords: grooming behavior, honey bee health, *Varroa*-resistance, hygienic behavior, natural selection, breeding programs

INTRODUCTION

The Western honey bee, *Apis mellifera* (Linnaeus), is one of the most valuable pollinators worldwide (Aizen and Harder, 2009; Gallai et al., 2009; Hung et al., 2018), providing essential pollination services to agroecosystems as well as profitable hive products for the apicultural sector (Morse and Calderone, 2000; Klein et al., 2007). Over the last few decades, honey bee colony losses have increased dramatically, as reported mainly in the Northern Hemisphere (Neumann and Carreck, 2010; Potts et al., 2010b; vanEngelsdorp et al., 2011), but also in South Africa (Pirk et al., 2014), Oceania (Brown et al., 2018), and South America (Vandame and Palacio, 2010; Maggi et al., 2016; Antúnez et al., 2017; Requier et al., 2018). The possible driving factors of these losses include a growing number of interacting threats, such as environment and climate change, nutritional deficiencies, pesticides, parasites, and pathogens (reviewed by Le Conte and Navajas, 2008; Potts et al., 2010a; Goulson et al., 2015).

The mite *Varroa destructor* (Anderson and Trueman) is considered the main parasite threatening *A. mellifera* colony survival, mainly in honey bee populations of European origin (Rosenkranz et al., 2010). Although the mite does not directly kill the bees, it has strong effects by weakening brood and adults through feeding on them (Amdam et al., 2004; Zaobidna et al., 2017; Ramsey et al., 2019) and transmitting several honey bee viruses (Dainat and Neumann, 2013; Francis et al., 2013; Mondet et al., 2014; McMenamin and Genersch, 2015). Together, these effects can affected the wing development and shorten the life span of worker bees and generate an epidemic disease within the colony, eventually resulting in colony death (Boecking and Genersch, 2008; Neumann et al., 2012; Van Dooremalen et al., 2012).

Today, most managed *A. mellifera* colonies depend on mite control treatments to survive (Rosenkranz et al., 2010). However, several *Varroa*-surviving honey bee populations have been documented around the world as a result of selective breeding or natural selection (e.g., Locke, 2016; Le Conte and Mondet, 2017). Bees may survive *Varroa* through the expression of resistance or tolerance traits. Resistance involves a reduction in *Varroa* growth, while tolerance reduces parasitic burden despite similar levels of *Varroa* growth (Schneider and Ayres, 2008; Kurze et al., 2016). Resistance or tolerance to *V. destructor* mites is a typical characteristic of Africanized bees from South and Central America (e.g., Martin and Medina, 2004; Mondragón et al., 2005; Rivera-Marchand et al., 2012). There are also accounts of *Varroa* resistant and tolerant European-derived *A. mellifera* populations from North America, Europe, and other parts of the world (e.g., Fries et al., 2006; Le Conte et al., 2007; Seeley, 2007; Pritchard, 2016). Specific adaptive behaviors have evolved in these honey bee populations, mainly related to resistance mechanisms, such as hygienic behavior specifically targeting *Varroa*-infested capped brood cells (VSH), recapping, and grooming (reviewed by Mondet et al., 2020).

Grooming behavior involves the physical removal, and often injury, of parasitic mites from the body of adult bees by individual workers or by their nest-mates. Through this behavior, the parasitized bees can dislodge mites themselves using their legs and mandibles (autogrooming) or receiving help from other bees (allogrooming) (Boecking and Spivak, 1999). Grooming is one of the main mechanisms of resistance against mite in *A. cerana* (Peng et al., 1987), and it is also observed in *A. mellifera* but expressed at a lower frequency (Boecking and Ritter, 1993; Fries et al., 1996). Despite these observations, several studies have evidenced that grooming behavior confers some degree of resistance against the *Varroa* mite in populations of Africanized bees (Moretto et al., 1993; Guzman-Novoa et al., 1999, 2002; Arechavaleta-Velasco and Guzmán-Novoa, 2001; Martin and Medina, 2004; Mondragón et al., 2005, 2006; Invernizzi et al., 2015). In European races of honey bees, grooming effectiveness against varroa mites is still unclear, although phenotypic variation for this behavior has been documented (Moosbeckhofer, 1997; Currie and Tahmasbi, 2008; Andino and Hunt, 2011; Bąk and Wilde, 2015). Guzman-Novoa et al. (2012) compared mite-resistant and susceptible honey bee stocks of different origins (Africanized bees, Russian, and European races) and found that all resistant stocks showed comparatively higher proportions of injured mites falling from colonies and increased intensity of individual grooming actions in laboratory assays, which underscores the importance of this trait in *Varroa* resistance. In fact, higher proportions of mite injuries inflicted by grooming have been associated with decreases in mite infestation levels observed in *A. mellifera* colonies (Moosbeckhofer, 1992; Arechavaleta-Velasco and Guzmán-Novoa, 2001; Mondragón et al., 2005; Guzman-Novoa et al., 2012).

Over the last three decades, numerous breeding programs have been initiated to selectively enhance heritable resistance or tolerance to the mite on specific honey bee populations (reviewed by Guichard et al., 2020). Such developments relied on the identification of specific traits that characterize these populations. This is a critical point since some characteristics that strongly confer mite resistance to some bee populations may not have a great influence on others (Locke, 2016). In Argentina, efforts have been made to identify and select local stocks that survive without mite treatment and characterize the underlying mechanisms. One of the criteria used by local breeding programs is the selection of hygienic behavior. This behavior involves

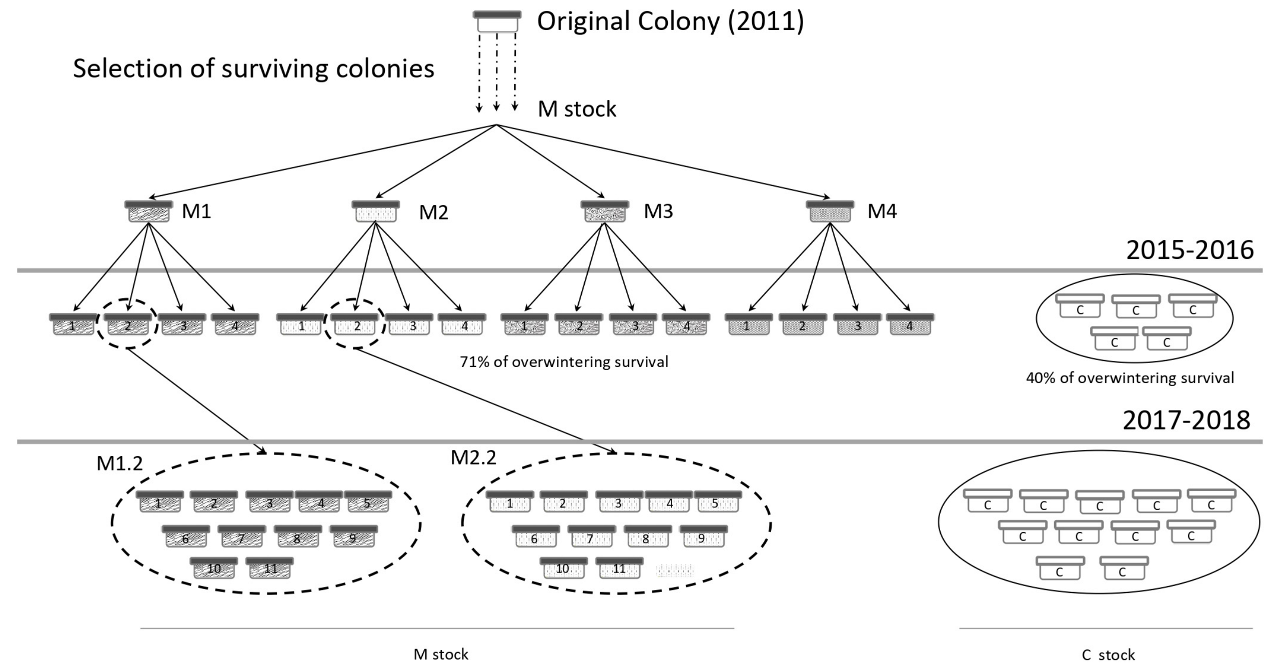

FIGURE 1 | Selection scheme performed to obtain the *Varroa*-surviving honey bee stock (M) and experimental design of the present survey (2017–2018 season). C corresponds to control colonies.

the workers' detection, uncapping and removal of unhealthy or dead brood (Rothenbuhler, 1964). Based on the pin-killed brood method, Argentinian honey bee populations have been studied and selected (Palacio et al., 2000, 2010). These honey bee stocks were later evaluated in relation to *Varroa* resistance in regions of temperate climate, where the mite has become a serious problem (Merke, 2016; Visintini, 2018). However, the phenotypic variation of grooming behavior and its contribution to colony survival has not been previously addressed in *Varroa*-resistant stocks from the country.

The objectives of this study were to characterize a *Varroa*-surviving honey bee stock located in North-Central Argentina, a region with a subtropical climate, and to evaluate the contribution of grooming behavior to mite-resistance. The integral characterization of this naturally selected honey bee population and the associated varroa mite provides a better understanding of the adaptive ways in which honey bee colonies can respond to mite infestation. Our results contribute to enhancing the management and breeding strategies for regional apiculture.

MATERIALS AND METHODS

Colonies for the Present Survey

A. mellifera colonies from two stocks were sampled: (1) a *Varroa*-surviving honey bee population (M, 22 colonies), and (2) a susceptible honey bee population (considered a control to our assays, C, 11 colonies) located at the apiary of Reconquista Agricultural Experimental Station (EEA Reconquista, 29°15′31.8″S 59°44′36.0″W) of the National Institute of Agricultural Technology (INTA). They were surveyed during the 2017–2018 season (**Figure 1**). This region is defined as Transition Chaco and characterized by a subtropical climate with a dry season. The control population was chosen for its geographical sympatry with the surviving population. Control colonies were headed by commercial queens of European origin and were known to require synthetic acaricide treatments against *V. destructor* twice a year (one in early autumn and one in early spring) or else suffer severe losses. A previous study (Russo et al., 2018) evidence 60% of overwinter colony mortality for this stock in absence of mite treatment. Colonies of both stocks received the same beekeeping practices and were not subjected to acaricide treatment during the survey.

Origin and Selection of the *Varroa*-Surviving Honey Bee Stock

The *Varroa*-surviving stock (M) is a honey bee population that had been kept without mite treatment for 6 years prior to the beginning of the present study (March 2017) (**Figure 1**). This stock was derived from a single colony from an abandoned commercial apiary at Reconquista locality (north of Santa Fe province, Argentina), where most of the colonies had died. The surviving colony was transported to the EEA Reconquista in 2011 and multiplied. Every spring, daughter colonies that survived winter without *Varroa* treatment and showed vitality in terms of colony growth were selected for the new generation. In the early spring of 2014, four colonies of M stock (named M1, M2, M3, and M4; **Figure 1**) were selected as mothers of the next generation and split into four new colonies each. The resulting sixteen daughter honey bee colonies were firstly monitored during the 2015–2016 season (**Figure 1**) and they showed higher overwintering survival and a higher proportion of fallen mites than the colonies from a commercial control stock (Russo et al., 2018). For the next generation, two colonies of M stock were selected as mothers and multiplied in 11 daughter colonies each to perform the present survey. During the selection process, queens of all colonies were naturally mated.

Genetic Characterization of Stocks

The mitochondrial (mt) haplotypes of all surveyed colonies were analyzed. Briefly, adult workers were collected from all colonies of M and C stocks during spring 2017. Total DNA was extracted from the thorax of one worker per colony following a high-salt protocol (Baruffi et al., 1995). DNA samples of honey bee workers were analyzed using a PCR-RFLP-based method. A fragment of 1,001 bp from the mitochondrial COI-COII region was amplified by PCR using the primers and conditions described by Hall and Smith (1991) and Lobo Segura (2000). PCR products were digested with *Hin*fI (Promega, Madison, MN, United States) following the manufacturer's recommendations. The restriction fragments were separated on 4% (wt/v) agarose gels, stained with GelRed, and photographed under UV light. The mt haplotypes detected in the restriction analysis using *Hin*fI were assigned as previously described by Agra et al. (2018).

Parameters Measured During the Survey

During the 2017–2018 season, the experimental apiary at EEA Reconquista was visited once in March 2017 (early autumn) and monthly during the active season, from September 2017 (early spring) to February 2018. During the visits, the following measurements were registered in each colony from both stocks (M and C): populations of adult bees and brood, percentage of mite infestation of adult bees, number of naturally fallen mites, and number of damaged mites. Overwintering survival of each stock was also registered. In addition, hygienic behavior and percentage of mite infestation on brood were measured twice, in March 2017 and September 2017.

Overwintering Colony Survival and Bee Population

The number of colonies that survived the winter was registered in spring (October 2017) for both stocks. Adult and brood populations were assessed in each colony by estimating the total area of comb covered by adult bees and brood according to DeGrandi-Hoffman et al. (2008). Briefly, once each hive was opened, frames were sequentially removed, and the percentage of the comb surface covered by adults and brood on both sides were registered. Then, the number of total frames fully occupied by adults and brood was estimated for each colony. The total number of bees per colony (total worker population) was estimated according to Delaplane et al. (2013).

Phoretic and Brood Infestation

The percentage of phoretic *Varroa* was determined by collecting and examining samples of approximately 300 workers from each colony. The samples were taken from the three central frames of each hive, by collecting the bees in plastic flasks previously filled with 70% ethanol. The number of mites detected in each sample was divided by the number of bees in the sample and multiplied by 100 to obtain the percentage of phoretic *Varroa* (De Jong et al., 1982). The total phoretic mite population was estimated for each colony using the percentage of phoretic *Varroa* and the estimated total worker population.

In addition, the percentage of mite infestation on brood was assessed once in autumn (March) and once in spring (October) 2017. Briefly, in each colony from both stocks, a frame with recently sealed brood (pupae not older than the purple- to dark-purple-eye stage) was identified. Fifty sealed brood cells from each side of the frame (a total of 100 cells per colony) were randomly selected and examined for the presence of adult female mites (Branco et al., 2006). The percentage of mite infestation on brood was the number of mite-infested cells.

Grooming Behavior

Grooming behavior was estimated by registering the mite fall and the damaged mites (indirect measures of grooming) (Boecking and Spivak, 1999). To this end, the screen bottom board method described by Pettis and Shimanuki (1999) was used. The original bottom board of each colony was replaced with a screened bottom board, allowing only the mites to fall through it and onto the slide-out inspection board. Before each measurement, the slide board of each colony was removed, cleaned, and reintroduced. Forty-eight hours later, the sliding boards were pulled out and the fallen mites were collected from the debris using a fine hairbrush. All fallen mites from each colony were counted and examined under a stereoscopic magnifying glass. Each mite received a binary score of "undamaged" or "damaged" for the analysis. In these cases, damage to the dorsal shield, gnathosoma, and legs was identified according to Rosenkranz et al. (1997) and Corrêa-Marques et al. (2000). The proportion of damaged mites in each colony was obtained by dividing the number of damaged mites by the total number of fallen mites collected at the end of the collection period (48 h). The proportion of fallen mites was obtained by dividing the number of fallen mites by the estimated total *Varroa* population of each colony, which represents the fraction of the mite removed by honey bees off their bodies relative to the total mite population present in the colonies.

Hygienic Behavior

Hygienic behavior was measured using the pin-killed brood assay (Newton and Ostasiewski, 1986; Palacio et al., 2000). Briefly, one frame of each colony containing a uniform capped brood was selected. On each frame, capped brood cells contained in a 10 × 5 cm comb section were perforated using an entomological pin (No. 1) to kill the brood. The frames were reintroduced in the original colony and inspected 24 h later to count the number of cells that had been cleaned by the bees. The hygienic activity of the colony was determined using the following equation:

$$HB\% = \left(\frac{\begin{array}{l} \textit{Total pin killed capped cells} \\ \quad -\textit{remaining capped cells} \\ \quad -\textit{uncapped cells with dead broad inside} \end{array}}{\textit{Total pin killed sealed cells}} \right) \times 100$$

Statistical Analysis

Overwintering colony survival was compared between stocks using a contingency-table analysis. To investigate whether adult bee population (number of frames fully occupied by bees), brood population (number of frames fully occupied by brood), and the percentage of phoretic *Varroa* differed between stocks and months across the season, separate generalized linear models (GLM) were performed including stock (M, C) and months of the active season (March, September, October, December, January, February) as fixed factors, and colonies as random factors. Logit transformation (ln) was applied to phoretic *Varroa* data. Similarly, the percentage of hygienic behavior was compared between stocks (M, C) and seasons (early autumn, early spring) by using GLM. Multiple comparisons were performed using Fisher LSD ($\alpha = 0.05$) in all cases.

Fallen mites and damaged mites were analyzed separately by using the general linear mixed model (GLMM) with a binomial distribution and logit link function (fallen vs. not fallen mites and damaged vs. undamaged mites, respectively) considering stocks and months, as fixed factors, and colonies, as a random factor. In the case of damaged mites, the comparisons between months were performed separately for each stock to obtain a better adjustment to the model. Multiple comparisons were performed using Fisher LSD ($\alpha = 0.05$). In all cases (GLMs and GLMMs), the Shapiro-Wilks and Levene tests and the residue normality were analyzed. To obtain the most appropriate structure of variance, the Akaike information criterion was used.

In addition, to find relationships between the measurements of grooming behavior and phoretic infestation of adults, the percentages of fallen mites, damaged mites, and phoretic mites were subjected to Spearman Rank Correlation analysis for each stock.

Possible differences in the types of damage on fallen mites from C and M colonies were analyzed with contingency tables. Specifically, the frequency of different categories of damage described above (legs, dorsal shield, gnathosoma) and the frequencies of multiple (legs + body) vs. simple (legs or body) damage were considered.

The frequencies of mite infestation on brood were compared between stocks by using contingency-table analysis. All statistical analyses were performed using InfoStat (Di Rienzo and Montiglio, 2016).

RESULTS

The genetic characterization of honey bee colonies used in the present study showed the presence of 100% of European haplotypes (C1) in both M and C colonies.

The percentage of overwintering survival (March to September 2017) was higher for M (81.8%) than for C (45.4%) stock [$\chi^2_{(1)} = 4.59$, $P = 0.032$]. The adult bee population across the active season was similar between C and M stocks with a significant difference only in early spring [GLMM results: $F_{(1,31)} = 0.01$, $P = 0.92$ for stock; $F_{(5,105)} = 24.45$, $P < 0.001$ for month; $F_{(5,105)} = 2.59$, $P = 0.03$ for interaction stock × month; *post hoc* comparisons in **Figure 2A**]. Specifically in September, the mean number of frames completely covered by bees was higher in M (6.76 ± 0.55) than in C (5.05 ± 0.98) (**Figure 2A**). Within C stock, the adult bee population was significantly lower in spring (September: 5.5 ± 0.9, and October: 5.8 ± 0.9) than in the other months evaluated (mean value: 8.6 ± 0.3), while no significant differences in this variable was detected across the season for M colonies (mean value: 7.4 ± 0.4) (**Figure 2A**).

Regarding the estimated brood population, variation in the number of frames occupied by brood across the season was detected for both stocks [GLMM results: $F_{(1,52)} = 2.91$, $P = 0.09$ for stock; $F_{(5,76)} = 20.01$, $P < 0.001$ for month; $F_{(5,76)} = 4.47$, $P < 0.01$ for interaction stock × month; **Figure 2B**]. Though similar brood patterns were observed between stocks in most monitored months, a significant difference was detected between M and C colonies for the mean number of frames with brood in early spring (September: M = 4.35 ± 0.23; C = 3.20 ± 0.45; **Figure 2B**).

According to the population dynamics of the colonies, the percentage of phoretic *Varroa* varied throughout the active season [GLMM results: $F_{(1,31)} = 2.66$, $P = 0.11$ for stock; $F_{(5,73)} = 22.94$, $P < 0.001$ for month; $F_{(5,73)} = 2.40$, $P = 0.049$ for interaction stock × month, **Figure 3A**]. The dynamics of phoretic infestation was similar in M and C colonies, with growing levels from spring to summer, and a peak in early autumn (**Figure 3A**). The increase in the percentage of phoretic *Varroa* observed in March was significantly higher for C (5.2 ± 1.2) than for M colonies (11.5 ± 1.7) (**Figure 3A**).

Consistent with the results of phoretic *Varroa*, the percentage of mite infestation on brood was significantly higher in C (6.64%) than in M (2.57%) in early autumn [$\chi^{2(1)} = 31.28$, $P < 0.001$; **Figure 3B**]. An extremely low percentage of infestation on brood was evident in both M and C stocks during spring [0.09% in M and 0% in C; $\chi^{2(1)} = 1.05$, $P = 0.31$; **Figure 3B**].

Hygienic behavior (HB) differed between stocks and seasons [GLMM results: $F_{(1,29)} = 5.64$, $P = 0.02$ for stock; $F_{(1,16)} = 11.21$, $P = 0.004$ for season; $F_{(1,16)} = 3.79$, $P = 0.07$ for interaction stock × season]. Specifically for M stock, HB was similar between spring and autumn, while for C stock, a lower level of HB was observed in autumn than in spring (**Table 1**). M showed higher HB than C only in autumn (**Table 1**).

The percentage of fallen mites on bottom boards differed between stocks and months, with a significant interaction between factors [GLMM results: $F_{(1,127)} = 23.67$, $P < 0.001$ for stock; $F_{(5,127)} = 16.16$, $P < 0.001$ for month; $F_{(5,127)} = 12.55$, $P < 0.001$ for interaction stock × month; **Figure 4A**]. Significant variation in this variable was detected across the season for M colonies, with September and February being the months with the highest mite fall percentage (*post hoc* comparisons by Fisher LSD, **Figure 4A**). Conversely, C colonies evidenced a low percentage of fallen mites without significant differences across the season (**Figure 4A**).

The mean percentage of damaged mites over the season was higher in M (25%) than in C (9%) stock [$F_{(1,97)} = 8.51$, $P < 0.01$]. C colonies exhibited a very low and similar number of damaged mites across the season [$F_{(1,16)} = 0.01$, $P > 0.05$; **Figure 4B**]. Conversely, this parameter varied throughout the season for M line [$F_{(1,71)} = 5.18$, $P < 0.001$; **Figure 4B**] with relatively greater

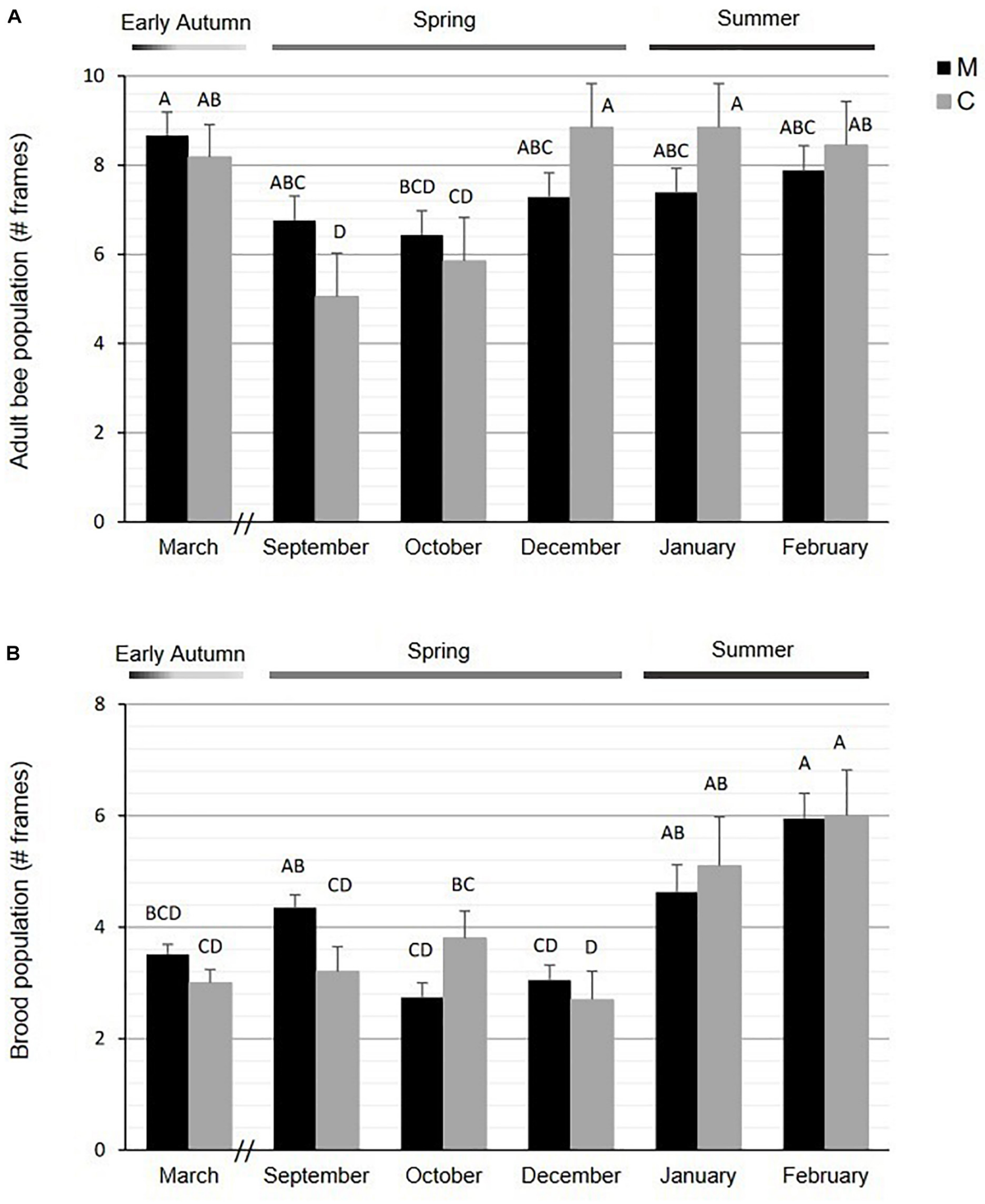

FIGURE 2 | Mean number (± SE) of frames fully occupied by **(A)** adult bees and **(B)** brood in M and C stocks. Different letters indicate significant differences in *post hoc* comparisons by Fisher LSD (α = 005).

damage during spring (September and October) and end of summer (February) (**Figure 4B**).

An association between the percentage of fallen mites and that of damaged mites was evident for M (Spearman's rank correlation: $r = 0.45$, $P < 0.001$) but not for C colonies. No association was found between the percentage of phoretic *Varroa* and grooming parameters (fallen and damaged mites) in M stock. Conversely, a positive correlation was detected between the percentage of phoretic *Varroa* and that of fallen mites in C stock (Spearman's rank correlation: $r = 0.4$, $P < 0.05$).

Different categories of damage to the mite were recorded in this study (**Figures 5A–F**). These categories were present in mites from colonies of both stocks, but at different relative frequencies depending on the colony origin (**Table 2**). Damaged leg (total or partial loss of one or more legs) was the predominant type of physical injury to the mite recorded in both M and C lines in similar percentages (**Table 2**), but with different intensity. In fact, significant differences were detected in the proportion of mites that presented more than 2 damaged legs in M (63.3%) than in C (10.5%) stock [$\chi^{2(1)} = 20.98$,

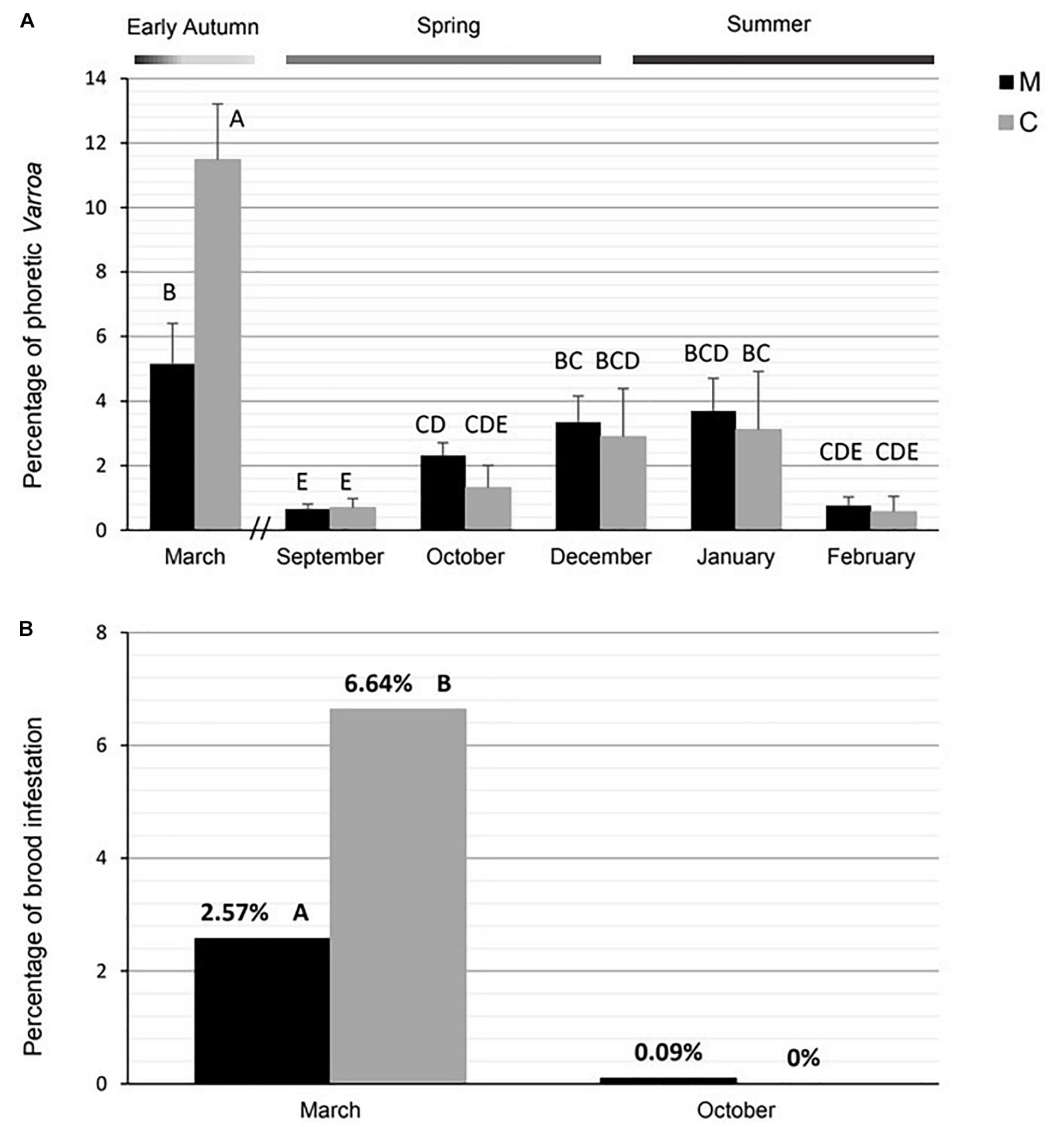

FIGURE 3 | Mean percentage (± SE) of **(A)** phoretic *Varroa* and **(B)** brood infestation in M and C stocks. Different letters indicate significant differences between stocks or months in **(A)** *post hoc* comparisons by Fisher LSD or **(B)** contingency analysis.

TABLE 1 | Percentage of hygienic behavior (±SE) measured in early autumn (March) and spring (October) for M and C stocks.

	C	M
Autumn	68.7 (3.9) B	82.4 (3.0) A
Spring	89.8 (5.7) A	88.8 (3.4) A

Different letters indicate significant differences between stocks or months by Fisher LSD (α = 0.05).

$P < 0.001$; **Supplementary Table S1**]. Moreover, 52.7% of the damaged mites from M colonies presented 4 or more damaged legs (**Supplementary Table S1**). Combined injury in body and legs (damaged legs + damaged gnathosoma or dorsal shield) was detected in 20.6 and 5% of the injured mites from M and C colonies, respectively. This difference was marginally significant (**Table 2**).

DISCUSSION

Here, we present a field survey of a naturally mite-surviving honey bee stock from north-east Argentina and explore the contribution of grooming behavior and colony dynamic to *Varroa*-resistance.

Our findings revealed that the *Varroa*-surviving honey bee stock (M) expressed a higher grooming behavior than that of the susceptible local control stock (C). This was evident in the higher mite damage recorded on the screened

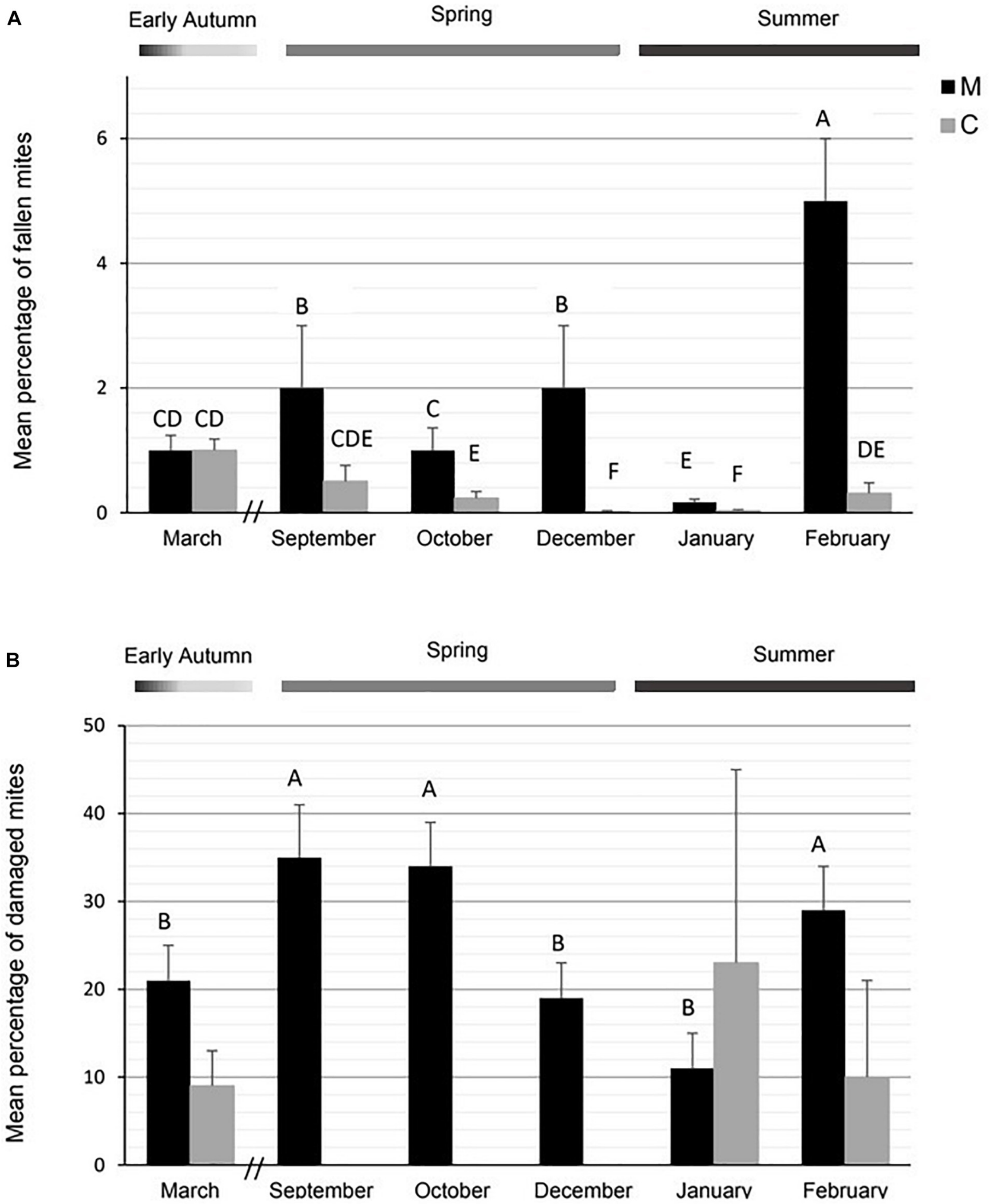

FIGURE 4 | Mean percentage (± SE) of **(A)** fallen mites and **(B)** damaged mites in M and C stocks. Different letters indicate significant differences in GLMM between stocks or months by Fisher LSD (0.05). No significant differences were detected in the percentage of damaged mites between months for C stock.

bottom boards of M compared to C colonies. The mean percentage of mite damage observed in our M population during spring (34.5%) appeared to be intermediate between those recorded in *A. m. ligustica* (26.4%) by Fries et al. (1996) and in Africanized *A. mellifera* (38.5%) by Moretto et al. (1991). We also detected phenotypic variation among stocks for the percentage of mite infestation in adults (phoretic *Varroa*) and in brood, particularly in early autumn. At this time of the season, C stock evidenced 2.2 and 2.6-fold more mites in adults and brood, respectively, compared to M stock. The difference in *Varroa* infestation between stocks in early autumn appeared to largely impact on the

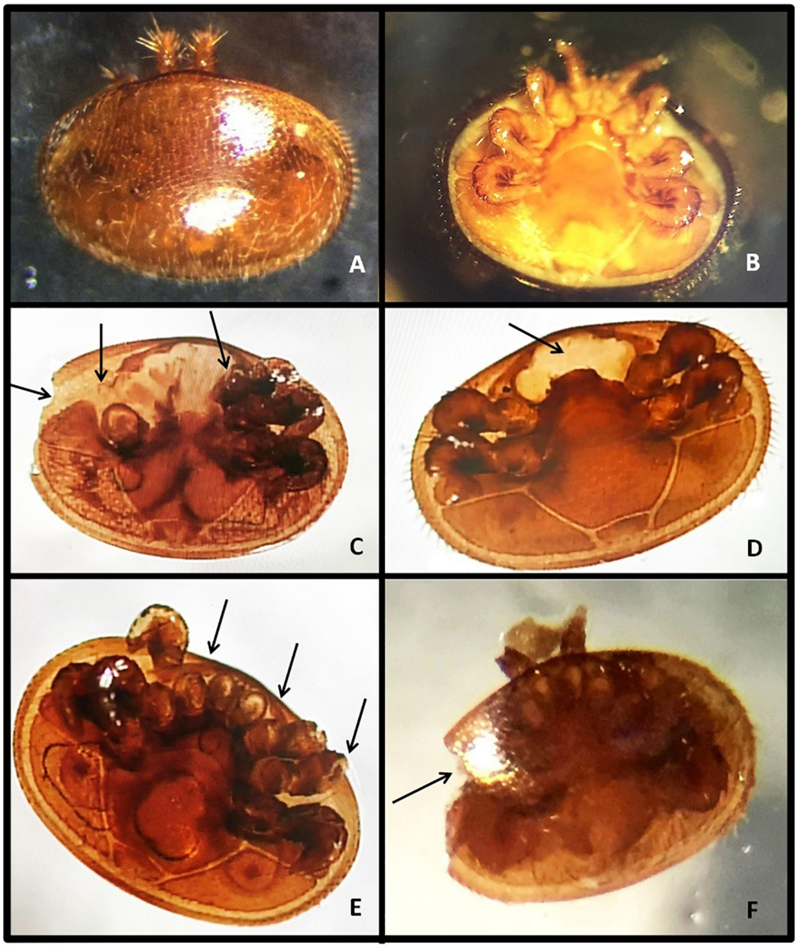

FIGURE 5 | Photographs showing the different damage patterns in *V. destructor* mites. Arrows indicate the injuries on mite's body, legs, and chelicerae. **(A,B)** Dorsal and ventral views of non-damaged mites, **(C)** damaged dorsal shield + damaged gnathosoma, and missing legs + chelicerae, **(D,E)** missing legs + chelicerae, **(F)** damaged dorsal shield + damaged gnathosoma. Classification of damage to the mites was previously reported by Corrêa-Marques et al. (2000). All the pictures were taken with an Olympus BX40 Microscope at 40x magnification.

TABLE 2 | Mean percentages for the different categories of damage to *V. destructor* recorded in the colony debris of M and C stocks.

Category of damage	C (%)	M (%)	*P*
Damaged legs (DL)	70	66.6	0.123
Damaged dorsal shield (DDS)	15	2.3	**0.001**
Damaged gnathosoma (DG)	10	8.5	0.172
Damaged body (DB)	0	2.0	0.369
Multiple damage (MD)	5	20.6	0.062

DL includes total or partial loss of one or more legs. DDS includes partial loss of dorsal shields and/or the presence of fissures; DG includes loss of chelicerae and/or pedipalps; DB includes DG + DDS; MD includes mites with DL + DG + DDS. P-values (after Bonferroni correction for multiple comparisons) from χ^2*-tests are shown. Significant comparisons (*$P < 0.05$*) are in bold.*

observed overwintering survival and the colony strength at the beginning of the season.

The high percentage of damaged mites observed during the active season and the more intense injuries inflicted on the mites by M bees, as discussed in more detail below, suggest that grooming behavior could increase mite mortality and hence modulate its population growth in the colonies. Our results are in line with a growing body of evidence (Morse et al., 1991; Moosbeckhofer, 1992; Ruttner and Hänel, 1992; Boecking and Ritter, 1993; Moretto et al., 1993; Bienefeld et al., 1999; Arechavaleta-Velasco and Guzmán-Novoa, 2001; Guzman-Novoa et al., 2012; Invernizzi et al., 2015; Nganso et al., 2017; Russo et al., 2018) suggesting that grooming behavior may be an important mechanism conferring resistance to honey bee colonies toward *V. destructor*, even in honey bee populations of European origin. Moreover, this trait may evolve by natural selection (as shown by the present results) and can be further developed or improved in ongoing selected stocks (e.g., Hunt et al., 2016).

The percentage of damaged mites showed seasonal variation, as previously suggested for grooming behavior (Büchler, 1994; Moosbeckhofer, 1997). Specifically, M colonies exhibited a high percentage of damaged mites during spring, where the phoretic infestation is low, in agreement with previous observations by

Mondragón et al. (2005). In this sense, M (in contrast to C) colonies may strategically respond to mite phoretic infestation below a load threshold, slowing the population growth of the mite and ensuring fewer loads to deal with overwintering. Even though Kruitwagen et al. (2017) suggested that grooming would not be mite-density dependent and speculated that it would only be beneficial at high levels of mite infestation, this pattern was specifically observed in control colonies (like our C colonies) and at a small mite infestation range. On the one hand, our results can be used to recommend specific times of the season to measure and select the grooming behavior performance at the colony level. On the other hand, our results are in line with observations on colonies bred for hygienic behavior, which are more efficient at removing *Varroa*-infested brood only under low mite parasitism (Spivak and Reuter, 1998, 2001; Ibrahim and Spivak, 2006). This hypothesis must be further evaluated in controlled assays that test the response of groomer colonies against different parasite loads.

As the proportion of damaged mites can be a time-consuming measurement in field surveys (Rosenkranz et al., 1997; Bienefeld et al., 1999; Aumeier, 2001), it has been suggested that mite fall could be a simpler alternative to select the grooming behavior of a colony (e.g., Kruitwagen et al., 2017). The present results evidenced a positive correlation between the percentage of fallen and damaged mites in M stock, but not in C stock. Therefore, the validity of using only the percentage of mite fall as a measure to select colonies for increasing grooming abilities must be further evaluated on different stocks, specifically if the selection is initiated on a honey bee population or is used to increase this trait in already groomer stock (as in the present case). For unselected stocks, as our C colonies, the mite fall may reflect mainly the passive fall of the mite [consistent with its use as an estimator of mite infestation at colony level (Branco et al., 2006; Guzman-Novoa et al., 2012; Hunt et al., 2016)], and it may not strictly represent a measure of grooming activity by the adult bees.

To characterize the differences in grooming behavior intensity between the stocks, we analyzed the patterns of damage in mites using the known classification performed by Corrêa-Marques et al. (2000). In agreement with previous studies (Ruttner and Hänel, 1992; Lodesani et al., 1996; Rosenkranz et al., 1997; Corrêa-Marques et al., 2000; Stanimirovic et al., 2003), we found that leg damage was the most frequent damage in mites from colonies of both origins. While the percentage of this kind of damage did not differ between M and C colonies, the number of damaged legs was higher in mites of M colonies than C colonies. In fact, more than 50% of the mites from M colonies evidenced damage in 4 or more legs. This, together with an apparent higher frequency of multiple injuries (legs and gnathosoma or dorsal shield damage) to the mites from M stock, would reflect that more intense grooming, possibly collective behavior (allogrooming), was displayed by adult bees from this origin. Accordingly, Nganso et al. (2017) detected the same kind of combined injuries to mites from both African and European honey bee colonies, but at higher frequency in the former. Overall, the high rate of mite mutilations observed in our M stock reflects how robust is the mite damage as indirect measurement of grooming behavior at the colony level. Even this measurement is tedious and time-consuming in field surveys, it is the only reliable phenotypic trait to breed for increased grooming behavior so far known. Alternatively, the mite population growth, estimated by determining the difference between two measurements of mite fall assessments over time, could represent a simpler and less time-consuming method to predict *Varroa*-resistance in honey bee populations (Emsen et al., 2012) since it may estimate several mechanisms of mite resistance simultaneously (e.g., grooming behavior, VSH, etc.).

Despite the European mitochondrial lineage of our stocks, as the analyzed region represents a hybrid zone where Africanized and European honey bee populations coexist (Agra et al., 2018), our stock may be a local ecotype that carries genes from both origins. In fact, we observed differences in the temperament of the stocks during field monitoring, with more excitable behavior in M than in C workers. Consistently, previous studies revealed that subspecies of *A. mellifera* described as more excitable or even aggressive differed from other subspecies in their grooming behavior in laboratory assays (Aumeier, 2001; Wilde et al., 2003; Bąk and Wilde, 2015). Further laboratory assays on this stock will allow us to investigate the apparently greater intensity of the grooming reactions of M worker bees against *V. destructor* and to test the association between the proportion of damaged mites in field monitoring and the proportion of mites dislodged by the bees in lab grooming assays (as previously detected by Andino and Hunt, 2011; Guzman-Novoa et al., 2012; Invernizzi et al., 2015). Moreover, these experiments will enable us to elucidate the weight of individual (autogrooming) and social (allogrooming) responses in the behavioral resistance against *V. destructor* in this stock and the best parameters to quantify each response.

It is important to note that grooming behavior may not be the only sanitary trait involved in regulating *Varroa* parasitism in M colonies. In fact, this stock expressed a higher hygienic behavior toward dead brood than did the control stock during early autumn, when the percentage of brood infestation is high. This result suggests that the bees of the surviving stock display higher hygiene and can behaviorally respond to the infestation status of the colony. However, since the method used here to test hygienic behavior may overestimate the expression of this behavior (Espinosa-Montaño et al., 2008), these results have to be taken with caution and confirmed in future research using more reliable methods for testing this complex behavior. Additionally, although hygienic behavior against dead brood does not necessarily imply greater resistance to *Varroa* (e.g., Danka et al., 2013), it would be linked to other associated behaviors such as *Varroa* Sensitive Hygiene (VSH; Spivak, 1996; Visintini, 2018), which were not measured in this work. In this sense, the analysis of other host traits that can jointly determine the surviving phenotype of our M stock (as previously evidenced in other naturally surviving stocks: Fries et al., 2006; Harris et al., 2010; Locke and Fries, 2011; Panziera et al., 2017; Oddie et al., 2018) is needed.

In addition, analyzing the performance of these colonies under different environments may help to clarify the influence of genotype x environment interactions (Büchler et al., 2014; Meixner et al., 2014) on grooming. It must be noted that this behavior can be influenced by environmental factors (Stanimirovic et al., 2003; Currie and Tahmasbi, 2008) and that *Varroa* damage thresholds can change under different environmental conditions (Meixner et al., 2014; Giacobino et al., 2017). In this sense, the development of regional breeding programs for mite-resistant honey bees that take advantage of the locally-adapted stocks deserves consideration, especially in Argentina where contrasting eco-regions coexist.

Given the complexity of measuring the mite damage at the colony level (the best way to phenotype grooming behavior according to our results) and the efforts involved in selecting it at a large scale, the genetic characterization of M stock would facilitate the identification of candidate genes associated with this trait. In turn, this identification would help to further develop marker-assisted selection tools for facilitating breeding efforts (Grozinger and Robinson, 2015; Guarna et al., 2017). Recent findings demonstrated a significant correlation between the expression of the gene *neurexin* and direct observations of grooming behavior (Hamiduzzaman et al., 2017). Furthermore, Morfin et al. (2019) found a correlation between this gene and mite mutilation, which reinforces the validity of analyzing mite damage as an indirect measurement of grooming behavior until the development of robust markers for marker-assisted selection. Finally, efforts are being made to characterize the productivity of the selected stock under standard beekeeping management. This information will greatly contribute to incorporating this genetic material into the breeding program conducted by INTA and to making it available for commercialization in the region.

CONCLUSION

Our data show that increased grooming behavior seems to be an important factor in reducing autumn *Varroa* infestation and enhancing overwintering survival of honey bee colonies of European origin, and suggest that mite damage would be the best proxy to evaluate and select this trait in the field. The characterization of this Argentinian stock, together with previously reported cases, clearly shows that honey bee populations can develop (different) traits and specific colony dynamics to overcome *V. destructor* infestations by means of natural selection. Taking advantage of these cases would be useful for a practical application in the apiculture and conservation of locally adapted honey bee populations.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

AS, JM, HP, SL, GR, and MP conceived and designed the research. RR, LL, HF, HP, JM, and IM conducted the experiments. ML contributed to statistical analyses. RR, SL, and AS analyzed the data and wrote the manuscript. All authors read and reviewed drafts of the manuscript and approved its final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2020.590281/full#supplementary-material

REFERENCES

Agra, M. N., Conte, C. A., Corva, P. M., Cladera, J. L., Lanzavecchia, S. B., and Palacio, M. A. (2018). Molecular characterization of *Apis mellifera* colonies from Argentina: genotypic admixture associated with ecoclimatic regions and apicultural activities. *Entomol. Exp. Appl.* 166, 724–738. doi: 10.1111/eea.12719

Aizen, M. A., and Harder, L. D. (2009). The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. *Curr. Biol.* 19, 915–918. doi: 10.1016/j.cub.2009.03.071

Amdam, G. V., Hartfelder, K., Norberg, K., Hagen, A., and Omholt, S. W. (2004). Altered physiology in worker honey bees (*Hymenoptera*: *Apidae*) infested with the mite *Varroa destructor* (*Acari*: *Varroidae*): a factor in colony loss during overwintering? *J. Econ. Entomol.* 97, 741–747. doi: 10.1603/0022-0493(2004)097[0741:APIWHB]2.0.CO;2

Andino, G. K., and Hunt, G. J. (2011). A scientific note on a new assay to measure honeybee mite-grooming behavior. *Apidologie* 42, 481–484. doi: 10.1007/s13592-011-0004-1

Antúnez, K., Invernizzi, C., Mendoza, Y., and Zunino, P. (2017). Honeybee colony losses in Uruguay during 2013–2014. *Apidologie* 48, 364–370. doi: 10.1007/s13592-016-0482-2

Arechavaleta-Velasco, M. E., and Guzmán-Novoa, E. (2001). Relative effect of four characteristics that restrain the population growth of the mite *Varroa destructor* in honey bee (*Apis mellifera*) colonies. *Apidologie* 32, 157–174. doi: 10.1051/apido:2001121

Aumeier, P. (2001). Bioassay for grooming effectiveness towards *Varroa destructor* mites in Africanized and Carniolan honey bees. *Apidologie* 32, 81–90. doi: 10.1051/apido:2001113

Bąk, B., and Wilde, J. (2015). Grooming behavior by worker bees of various subspecies of honey bees to remove *Varroa destructor* mites. *J. Apic. Res.* 54, 207–215. doi: 10.1080/00218839.2016.1147791

Baruffi, L., Damiani, G., Guglielmino, C. R., Bandi, C., Malacrida, A. R., and Gasperi, G. (1995). Polymorphism within and between populations of *Ceratitis capitata*: comparison between RAPD and multilocus enzyme electrophoresis data. *Heredity* 74, 425–437. doi: 10.1038/hdy.1995.60

Bienefeld, K., Zautke, F., Pronin, D., and Mazeed, A. (1999). Recording the proportion of damaged *Varroa jacobsoni* Oud. in the debris of honey bee colonies (*Apis mellifera*). *Apidologie* 30, 249–256. doi: 10.1051/apido:19990401

Boecking, O., and Genersch, E. (2008). Varroosis–the ongoing crisis in bee keeping. *J. Verbr. Lebensm.* 3, 221–228. doi: 10.1007/s00003-008-0331-y

Boecking, O., and Ritter, W. (1993). Grooming and removal behaviour of *Apis mellifera* intermissa in Tunisia against *Varroa jacobsoni*. *J. Apic. Res.* 32, 127–134. doi: 10.1080/00218839.1993.11101297

Boecking, O., and Spivak, M. (1999). Behavioral defenses of honey bees against *Varroa jacobsoni* Oud. *Apidologie* 30, 141–158. doi: 10.1051/apido:19990205

Branco, M. R., Kidd, N. A., and Pickard, R. S. (2006). A comparative evaluation of sampling methods for *Varroa destructor* (*Acari*: *Varroidae*) population estimation. *Apidologie* 37, 452–461. doi: 10.1051/apido:2006010

Brown, P., Newstrom-Lloyd, L. E., Foster, B. J., Badger, P. H., and McLean, J. A. (2018). Winter 2016 honey bee colony losses in New Zealand. *J. Apic. Res.* 57, 278–291. doi: 10.1080/00218839.2018.1430980

Büchler, R. (1994). Varroa tolerance in honey bees-occurrence, characters and breeding. *Bee World* 75, 54–70. doi: 10.1080/0005772X.1994.11099201

Büchler, R., Costa, C., Hatjina, F., Andonov, S., Meixner, M. D., Conte, Y. L., et al. (2014). The influence of genetic origin and its interaction with environmental effects on the survival of *Apis mellifera* L. colonies in Europe. *J. Apic. Res.* 53, 230–232. doi: 10.3896/IBRA.1.53.2.03

Corrêa-Marques, M. H., Issa, M. R. C., and Jong, D. D. (2000). Classification and quantification of damaged *Varroa jacobsoni* found in the debris of honey bee colonies as criteria for selection? *Am. Bee J.* 140, 820–824.

Currie, R. W., and Tahmasbi, G. H. (2008). The ability of high-and low-grooming lines of honey bees to remove the parasitic mite *Varroa destructor* is affected by environmental conditions. *Can. J. Zool.* 86, 1059–1067. doi: 10.1139/Z08-083

Dainat, B., and Neumann, P. (2013). Clinical signs of deformed wing virus infection are predictive markers for honey bee colony losses. *J. Invertebr. Pathol.* 112, 278–280. doi: 10.1016/j.jip.2012.12.009

Danka, R. G., Harris, J. W., Villa, J. D., and Dodds, G. E. (2013). Varying congruence of hygienic responses to *Varroa destructor* and freeze-killed brood among different types of honeybees. *Apidologie* 44, 447–457. doi: 10.1007/s13592-013-0195-8

De Jong, D., Morse, R. A., and Eickwort, G. C. (1982). Mite pests of honey bees. *Annu. Rev. Entomol.* 27, 229–252. doi: 10.1146/annurev.en.27.010182.001305

DeGrandi-Hoffman, G., Wardell, G., Ahumada-Segura, F., Rinderer, T., Danka, R., and Pettis, J. (2008). Comparisons of pollen substitute diets for honey bees: consumption rates by colonies and effects on brood and adult populations. *J. Apic. Res.* 47, 265–270. doi: 10.1080/00218839.2008.11101473

Delaplane, K. S., Van Der Steen, J., and Guzman-Novoa, E. (2013). Standard methods for estimating strength parameters of *Apis mellifera* colonies. *J. Apic. Res.* 52, 1–12. doi: 10.3896/IBRA.1.52.4.12

Di Rienzo, N., and Montiglio, P. O. (2016). The contribution of developmental experience vs. condition to life history, trait variation and individual differences. *J. Anim. Ecol.* 85, 915–926. doi: 10.1111/1365-2656.12512

Emsen, B., Petukhova, T., and Guzman-Novoa, E. (2012). Factors limiting the growth of Varroa destructor populations in selected honey bee (*Apis mellifera* L.) colonies. *J. Anim. Vet. Adv.* 11, 4519–4525.

Espinosa-Montaño, L. G., Guzmán-Novoa, E., Sánchez-Albarrán, A., Montaldo, H. H., and Correa-Benítez, A. (2008). Comparative study of three assays to evaluate hygienic behavior in honey bee (*Apis mellifera* L.) colonies. *Vet. Mexico* 39, 39–54.

Francis, R. M., Nielsen, S. L., and Kryger, P. (2013). Varroa-virus interaction in collapsing honey bee colonies. *PLoS One* 8:e57540. doi: 10.1371/journal.pone.0057540

Fries, I., Huazhen, W., Wei, S., and Jin, C. S. (1996). Grooming behavior and damaged mites (*Varroa jacobsoni*) in *Apis cerana* cerana and *Apis mellifera* ligustica. *Apidologie* 27, 3–11. doi: 10.1051/apido:19960101

Fries, I., Imdorf, A., and Rosenkranz, P. (2006). Survival of mite infested (*Varroa destructor*) honey bee (*Apis mellifera*) colonies in a Nordic climate. *Apidologie* 37, 564–570. doi: 10.1051/apido:2006031

Gallai, N., Salles, J. M., Settele, J., and Vaissière, B. E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecol. Econ.* 68, 810–821. doi: 10.1016/j.ecolecon.2008.06.014

Giacobino, A., Pacini, A., Molineri, A., Cagnolo, N. B., Merke, J., Orellano, E., et al. (2017). Environment or beekeeping management: What explains better the prevalence of honey bee colonies with high levels of *Varroa destructor*? *Res. Vet. Sci.* 112, 1–6. doi: 10.1016/j.rvsc.2017.01.001

Goulson, D., Nicholls, E., Botías, C., and Rotheray, E. L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* 347:1255957. doi: 10.1126/science.1255957

Grozinger, C. M., and Robinson, G. E. (2015). The power and promise of applying genomics to honey bee health. *Curr. Opin. Insect. Sci.* 10, 124–132. doi: 10.1016/j.cois.2015.03.007

Guarna, M. M., Hoover, S. E., Huxter, E., Higo, H., Moon, K. M., Domanski, D., et al. (2017). Peptide biomarkers used for the selective breeding of a complex polygenic trait in honey bees. *Sci. Rep.* 7:8381. doi: 10.1038/s41598-017-08464-2

Guichard, M., Dietemann, V., Neuditschko, M., and Dainat, B. (2020). Three decades of selecting honey bees that survive infestations by the parasitic mite *Varroa destructor*: Outcomes, limitations and strategy. *Preprints* 2020, 1–83. doi: 10.20944/preprints202003.0044.v1

Guzman-Novoa, E., Emsen, B., Unger, P., Espinosa-Montaño, L. G., and Petukhova, T. (2012). Genotypic variability and relationships between mite infestation levels, mite damage, grooming intensity, and removal of *Varroa destructor* mites in selected strains of worker honey bees (*Apis mellifera* L.). *J. Invertebr. Pathol.* 110, 314–320. doi: 10.1016/j.jip.2012.03.020

Guzman-Novoa, E., Hunt, G. J., Page, R. E., and Fondrk, M. K. (2002). Genetic correlations among honey bee (Hymenoptera: Apidae) behavioral characteristics and wing length. *Ann. Entomol. Soc. Am.* 95, 402–406. doi: 10.1603/0013-8746(2002)095[0402:GCAHBH]2.0.CO;2

Guzman-Novoa, E., Vandame, R., and Arechavaleta, M. E. (1999). Susceptibility of European and Africanized honey bees (*Apis mellifera* L.) to *Varroa jacobsoni* Oud. in Mexico. *Apidologie* 30, 173–182. doi: 10.1051/apido:19990207

Hall, H. G., and Smith, D. R. (1991). Distinguishing African and European honeybee matrilines using amplified mitochondrial DNA. *Proc. Natl. Acad. Sci. U.S.A.* 88, 4548–4552. doi: 10.1073/pnas.88.10.4548

Hamiduzzaman, M. M., Emsen, B., Hunt, G. J., Subramanyam, S., Williams, C. E., Tsuruda, J. M., et al. (2017). Differential gene expression associated with honey bee grooming behavior in response to Varroa mites. *Behav. Genet.* 47, 335–344. doi: 10.1007/s10519-017-9834-6

Harris, J. W., Danka, R. G., and Villa, J. D. (2010). Honey bees (Hymenoptera: Apidae) with the trait of Varroa sensitive hygiene remove brood with all reproductive stages of Varroa mites (Mesostigmata: Varroidae). *Ann. Entomol. Soc. Am.* 103, 146–152. doi: 10.1603/AN09138

Hung, K. L. J., Kingston, J. M., Albrecht, M., Holway, D. A., and Kohn, J. R. (2018). The worldwide importance of honey bees as pollinators in natural habitats. *Proc. R. Soc. B.* 285:20172140. doi: 10.1098/rspb.2017.2140

Hunt, G., Given, J. K., Tsuruda, J. M., and Andino, G. K. (2016). Breeding mite-biting bees to control Varroa. *Bee Cult.* 8, 41–47.

Ibrahim, A., and Spivak, M. (2006). The relationship between hygienic behavior and suppression of mite reproduction as honey bee (*Apis mellifera*) mechanisms of resistance to *Varroa destructor*. *Apidologie* 37, 31–40. doi: 10.1051/apido:2005052

Invernizzi, C., Zefferino, I., Santos, E., Sánchez, L., and Mendoza, Y. (2015). Multilevel assessment of grooming behavior against *Varroa destructor* in Italian and Africanized honey bees. *J. Apic. Res.* 54, 321–327. doi: 10.1080/00218839.2016.1159055

Klein, A. M., Vaissiere, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., et al. (2007). Importance of pollinators in changing landscapes for world crops. *Proc. R. Soc. B.* 274, 303–313. doi: 10.1098/rspb.2006.3721

Kruitwagen, A., van Langevelde, F., van Dooremalen, C., and Blacquière, T. (2017). Naturally selected honey bee (*Apis mellifera*) colonies resistant to *Varroa destructor* do not groom more intensively. *J. Apic. Res.* 56, 354–365. doi: 10.1080/00218839.2017.1329797

Kurze, C., Routtu, J., and Moritz, R. F. (2016). Parasite resistance and tolerance in honeybees at the individual and social level. *Zoology* 119, 290–297. doi: 10.1016/j.zool.2016.03.007

Le Conte, Y., De Vaublanc, G., Crauser, D., Jeanne, F., Rousselle, J. C., and Bécard, J. M. (2007). Honey bee colonies that have survived *Varroa destructor*. *Apidologie* 38, 566–572. doi: 10.1051/apido:2007040

Le Conte, Y., and Mondet, F. (2017). "Natural selection of honey bees against *Varroa destructor*" in *Beekeeping–From Science to Practice*, eds R. H. Vreeland

and D. Sammataro (Cham: Springer), 189–194. doi: 10.1007/978-3-319-60637-8_12

Le Conte, Y., and Navajas, M. (2008). Climate change: impact on honey bee populations and diseases. *Rev. Sci. Tech. Off. Int. Épizoot.* 27, 499–510. doi: 10.20506/rst.27.2.1819

Lobo Segura, J. A. (2000). Highly polymorphic DNA markers in an Africanized honey bee population in Costa Rica. *Genet. Mol. Biol.* 23, 317–322. doi: 10.1590/S1415-47572000000200013

Locke, B. (2016). Natural Varroa mite-surviving *Apis mellifera* honeybee populations. *Apidologie* 47, 467–482. doi: 10.1007/s13592-015-0412-8

Locke, B., and Fries, I. (2011). Characteristics of honey bee colonies (*Apis mellifera*) in Sweden surviving Varroa destructor infestation. *Apidologie.* 42, 533–542. doi: 10.1007/s13592-011-0029-5

Lodesani, M., Vecchi, M. A., Tommasini, S., and Bigliardi, M. (1996). A study on different kinds of damage to *Varroa jacobsoni* in *Apis mellifera* ligustica colonies. *J. Apic. Res.* 35, 49–56. doi: 10.1080/00218839.1996.11100912

Maggi, M., Antúnez, K., Invernizzi, C., Aldea, P., Vargas, M., Negri, P., et al. (2016). Honeybee health in South America. *Apidologie* 47, 835–854. doi: 10.1007/s13592-016-0445-7

Martin, S. J., and Medina, L. M. (2004). Africanized honeybees have unique tolerance to Varroa mites. *Trends Parasitol.* 20, 112–114. doi: 10.1016/j.pt.2004.01.001

McMenamin, A. J., and Genersch, E. (2015). Honey bee colony losses and associated viruses. *Curr. Opin. Insect. Sci.* 8, 121–129. doi: 10.1016/j.cois.2015.01.015

Meixner, M. D., Francis, R. M., Gajda, A., Kryger, P., Andonov, S., Uzunov, A., et al. (2014). Occurrence of parasites and pathogens in honey bee colonies used in a European genotype-environment interactions experiment. *J. Apic. Res.* 53, 215–229. doi: 10.3896/IBRA.1.53.2.04

Merke, J. (2016). *Dinámica Poblacional de Varroa Destructor y Apis Mellifera L. Como Herramienta Para la Selección de Abejas Tolerantes*. Doc. Thesis, Argentina: University of Mar del Plata. .

Mondet, F., Beaurepaire, A., McAfee, A., Locke, B., Alaux, C., Blanchard, S., et al. (2020). Honey bee survival mechanisms against the parasite *Varroa destructor*: a systematic review of phenotypic and genomic research efforts. *Int. J. Parasitol.* 50, 433–447. doi: 10.1016/j.ijpara.2020.03.005

Mondet, F., de Miranda, J. R., Kretzschmar, A., Le Conte, Y., and Mercer, A. R. (2014). On the front line: quantitative virus dynamics in honeybee (*Apis mellifera* L.) colonies along a new expansion front of the parasite *Varroa destructor*. *PLoS Pathog.* 10:e1004323. doi: 10.1371/journal.ppat.1004323

Mondragón, L., Martin, S., and Vandame, R. (2006). Mortality of mite offspring: a major component of *Varroa destructor* resistance in a population of Africanized bees. *Apidologie* 37, 67–74. doi: 10.1051/apido:2005053

Mondragón, L., Spivak, M., and Vandame, R. (2005). A multifactorial study of the resistance of honeybees *Apis mellifera* to the mite *Varroa destructor* over one year in Mexico. *Apidologie* 36, 345–358. doi: 10.1051/apido:2005022

Moosbeckhofer, R. (1992). Observations on the occurrence of damaged Varroa mites in natural mite fall of *Apis mellifera* carnica. *Apidologie* 23, 523–531. doi: 10.1051/apido:19920604

Moosbeckhofer, R. (1997). Observations on reproduction rate of *Varroa jacobsoni* and the occurrence of mutilated mites in *Apis mellifera* carnica colonies. *Apidologie* 28, 193–195.

Moretto, G., Gonçalves, L. S., and De Jong, D. (1993). Heritability of Africanized and European honey bee defensive behavior against the mite *Varroa jacobsoni*. *Rev. Bras. Genét.* 16, 71–71.

Moretto, G., Gonçalves, L. S., De Jong, D., and Bichuette, M. Z. (1991). The effects of climate and bee race on *Varroa jacobsoni* Oud infestations in Brazil. *Apidologie* 22, 197–203. doi: 10.1051/apido:19910303

Morfin, N., Given, K., Evans, M., Guzman-Novoa, E., and Hunt, G. J. (2019). Grooming behavior and gene expression of the Indiana "mite-biter" honey bee stock. *Apidologie* 51, 267–275. doi: 10.1007/s13592-019-00710-y

Morse, R. A., and Calderone, N. W. (2000). The value of honey bees as pollinators of US crops in 2000. *Bee Cult.* 128, 1–15.

Morse, R. A., Miksa, D., and Masenheimer, J. A. (1991). Varroa resistance in US honey bees. *Am. Bee J.* 131, 433–434.

Neumann, P., and Carreck, N. (2010). Honey bee colony losses. *J. Apic. Res.* 49, 1–6. doi: 10.3896/IBRA.1.49.1.01

Neumann, P., Yañez, O., Fries, I., and de Miranda, J. R. (2012). Varroa invasion and virus adaptation. *Trends Parasitol.* 28, 353–354. doi: 10.1016/j.pt.2012.06.004

Newton, D. C., and Ostasiewski, N. J. Jr. (1986). A simplified bioassay for behavioral resistance to American foulbrood in honey bees. *Am. Bee J.* 126, 278–281.

Nganso, B. T., Fombong, A. T., Yusuf, A. A., Pirk, C. W., Stuhl, C., and Torto, B. (2017). Hygienic and grooming behaviors in African and European honeybees-New damage categories in *Varroa destructor*. *PLoS One* 12:e0179329. doi: 10.1371/journal.pone.0179329

Oddie, M., Büchler, R., Dahle, B., Kovacic, M., Le Conte, Y., Locke, B., et al. (2018). Rapid parallel evolution overcomes global honey bee parasite. *Sci. Rep.* 8:7704. doi: 10.1038/s41598-018-26001-7

Palacio, M. A., Figini, E. E., Ruffinengo, S. R., Rodriguez, E. M., del Hoyo, M. L., and Bedascarrasbure, E. L. (2000). Changes in a population of *Apis mellifera* L. selected for hygienic behaviour and its relation to brood disease tolerance. *Apidologie* 31, 471–478. doi: 10.1051/apido:2000139

Palacio, M. A., Rodriguez, E., Goncalves, L., Bedascarrasbure, E., and Spivak, M. (2010). Hygienic behaviors of honey bees in response to brood experimentally pin-killed or infected with *Ascosphaera Apis*. *Apidologie* 41, 602–612. doi: 10.1051/apido/2010022

Panziera, D., van Langevelde, F., and Blacquière, T. (2017). Varroa sensitive hygiene contributes to naturally selected Varroa resistance in honey bees. *J. Apic. Res.* 56, 635–642. doi: 10.1080/00218839.2017.1351860

Peng, Y. S., Fang, Y., Xu, S., and Ge, L. (1987). The resistance mechanism of the Asian honey bee, *Apis cerana* Fabr., to an ectoparasitic mite, *Varroa jacobsoni* Oudemans. *J. Invertebr, Pathol.* 49, 54–60. doi: 10.1016/0022-2011(87)90125-X

Pettis, J. S., and Shimanuki, H. (1999). A hive modification to reduce *Varroa* populations. *Am. Bee J.* 139, 471–473.

Pirk, C. W., Human, H., Crewe, R. M., and vanEngelsdorp, D. (2014). A survey of managed honey bee colony losses in the Republic of South Africa 2009 to 2011. *J. Apic. Res.* 53, 35–42. doi: 10.3896/IBRA.1.53.1.03

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., and Kunin, W. E. (2010a). Global pollinator declines: trends, impacts and drivers. *Trends Eco. Evol.* 25, 345–353. doi: 10.1016/j.tree.2010.01.007

Potts, S. G., Roberts, S. P., Dean, R., Marris, G., Brown, M. A., Jones, R., et al. (2010b). Declines of managed honey bees and beekeepers in Europe. *J. Apic. Res.* 49, 15–22. doi: 10.3896/IBRA.1.49.1.02

Pritchard, D. J. (2016). Grooming by honey bees as a component of Varroa resistant behavior. *J. Apic. Res.* 55, 38–48. doi: 10.1080/00218839.2016.1196016

Ramsey, S. D., Ochoa, R., Bauchan, G., Gulbronson, C., Mowery, J. D., Cohen, A., et al. (2019). *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. *Proc. Natl. Acad. Sci. U.S.A.* 116, 1792–1801. doi: 10.1073/pnas.1818371116

Requier, F., Antúnez, K., Morales, C. L., Aldea Sánchez, P., Castilhos, D., Garrido, P. M., et al. (2018). Trends in beekeeping and honey bee colony losses in Latin America. *J. Apic. Res.* 57, 657–662. doi: 10.1080/00218839.2018.1494919

Rivera-Marchand, B., Oskay, D., and Giray, T. (2012). Gentle Africanized bees on an oceanic island. *Evol. Appl.* 5, 746–756. doi: 10.1111/j.1752-4571.2012.00252.x

Rosenkranz, P., Aumeier, P., and Ziegelmann, B. (2010). Biology and control of *Varroa destructor*. *J. Invertebr. Pathol.* 103, S96–S119. doi: 10.1016/j.jip.2009.07.016

Rosenkranz, P., Fries, I., Boecking, O., and Stürmer, M. (1997). Damaged Varroa mites in the debris of honey bee (*Apis mellifera* L) colonies with and without hatching brood. *Apidologie* 28, 427–437. doi: 10.1051/apido:19970609

Rothenbuhler, W. C. (1964). Behaviour genetics of nest cleaning in honey bees. *I. Responses of four inbred lines to disease-killed brood. Anim. Behav.* 12, 578–583. doi: 10.1016/0003-3472(64)90082-X

Russo, R. M., Merke, J., Landi, L., Pietronave, H., Fain, H., Liendo, M. C., et al. (2018). *The Grooming Behavior as a Varroa-Tolerance Mechanism in Apis Mellifera Colonies from Argentina*. Guarujá: IUSSI.

Ruttner, F., and Hänel, H. (1992). Active defense against Varroa mites in a Carniolan strain of honeybee (*Apis mellifera* carnica Pollmann). *Apidologie* 23, 173–187. doi: 10.1051/apido:19920210

Schneider, D. S., and Ayres, J. S. (2008). Two ways to survive infection: what resistance and tolerance can teach us about treating infectious diseases. *Nat. Rev. Immunol.* 8, 889–895. doi: 10.1038/nri2432

Seeley, T. D. (2007). Honey bees of the Arnot Forest: a population of feral colonies persisting with *Varroa destructor* in the northeastern United States. *Apidologie* 38, 19–29. doi: 10.1051/apido:2006055

Spivak, M. (1996). Honey bee hygienic behavior and defense against *Varroa jacobsoni*. *Apidologie* 27, 245–260. doi: 10.1051/apido:19960407

Spivak, M., and Reuter, G. S. (1998). Performance of hygienic honey bee colonies in a commercial apiary. *Apidologie* 29, 291–302. doi: 10.1051/apido:19980308

Spivak, M., and Reuter, G. S. (2001). *Varroa destructor* infestation in untreated honey bee (Hymenoptera: Apidae) colonies selected for hygienic behavior. *J. Econ. Entomol.* 94, 326–331. doi: 10.1603/0022-0493-94.2.326

Stanimirovic, Z., Stevanovic, J., and Cirkovic, D. (2003). Investigations of reproductive, productive, hygienic and grooming features of Syenichko-Peshterski honey bee ecotype. *Apidologie* 34, 487–488.

Van Dooremalen, C., Gerritsen, L., Cornelissen, B., van der Steen, J. J., van Langevelde, F., and Blacquiere, T. (2012). Winter survival of individual honey bees and honey bee colonies depends on level of *Varroa destructor* infestation. *PLoS One* 7:e36285. doi: 10.1371/journal.pone.0036285

Vandame, R., and Palacio, M. A. (2010). Preserved honey bee health in Latin America: a fragile equilibrium due to low-intensity agriculture and beekeeping? *Apidologie* 41, 243–255. doi: 10.1051/apido/2010025

vanEngelsdorp, D., Hayes, J. Jr., Underwood, R. M., Caron, D., and Pettis, J. (2011). A survey of managed honey bee colony losses in the USA, fall 2009 to winter 2010. *J. Apic. Res.* 50, 1–10. doi: 10.3896/IBRA.1.50.1.01

Visintini, M. G. (2018). *Factores Relacionados con la Tolerancia Frente a Varroa Destructor de Colmenas de Apis Mellifera en Climas Templados*. Bach.Thesis degree, Universidad Nacional del Litoral: Argentina.

Wilde, J., Bratkowski, J., and Siuda, M. (2003). Defensive behavior of three breeds of *Apis mellifera* L. *Ann. Univ. Mariae Curie Sklodowska Sect. EE Zootech.* 21, 87–93.

Zaobidna, E. A., Żółtowska, K., and Łopieńska-Biernat, E. (2017). *Varroa destructor* induces changes in the expression of immunity-related genes during the development of *Apis mellifera* worker and drone broods. *Acta Parasitol.* 62, 779–789. oi: doi: 10.1515/ap-2017-0094

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Varroa destructor Parasitism and Genetic Variability at Honey Bee (*Apis mellifera*) Drone Congregation Areas and Their Associations With Environmental Variables in Argentina

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Varroa destructor is a serious ectoparasite of the western honey bee, *Apis mellifera*, which negatively impacts on colonies health and survival worldwide. Drone-mediated movement and the presence of the mite in Drone Congregation Areas (DCA) may play a relevant role in *Varroa* dispersal. The objectives of this study were to characterize mite infestation levels and genetic diversity in DCAs and surrounding apiaries and to explore putative environmental variables associated to *Varroa* infestation in two eco-climatic regions of Argentina (temperate, and subtropical). Phoretic mite proportions in DCAs and apiaries were estimated during spring. Landscape, topographic, and climate variables were described using satellite image classifications and data from public databases. The genetic composition of drones at the DCAs and workers from the surrounding apiaries was assessed using mitochondrial markers. In total, eleven DCAs were identified in both regions during 2017 and 2018. The mean proportion of *Varroa* was ca. 3 in 1,000 (0.0028 ± 0.0046) at the apiaries, and ca. 2 in 100 (0.0168 ± 0.0227) at the DCAs. No statistical differences were observed between apiaries and DCAs or between ecoregions, but the proportion of infested males at the DCAs was positively correlated to the distance to the apiary and a trend was observed toward higher mite loads in DCAs. Landscape and topography were not determinant for *Varroa* infestation at the DCAs but relative humidity and precipitation in the previous week of sampling, positively influenced infestation. More haplotypic diversity was detected in the DCAs compared to the surrounding apiaries, particularly in the subtropical region. While in this region high prevalence of Africanized (A1, A4) mitochondrial lineages was detected, European lineages (C1, C2j) were mostly found in apiaries and DCA in the temperate region. Our results provide valuable information on the dynamics of *Varroa* parasitism in apiaries and DCAs, and highlight the role of drones in mite dispersion and genetic variability of new colonies. The study of DCAs emerges as a tool for investigating not only honey bee reproduction and conservation, but also the impact of the environment on bee epidemiology.

Keywords: Argentina, DCA, eco-climatic regions, genetic variability, landscape metrics, mite parasitism

INTRODUCTION

Varroa destructor Anderson and Trueman (Acari: Varroidae) is considered the main ectoparasitic pathogen of the western honey bee *Apis mellifera* Linnaeus (Hymenoptera: Apidae) worldwide. No other pathogen has had a comparable impact on honey bee health and colony survival during the history of apiculture (Guzmán-Novoa et al., 2010; Le Conte et al., 2010; Neumann and Carreck, 2010; Francis et al., 2013). The dynamics of *V. destructor* populations and the impact of mite parasitism in honey bee colonies are influenced by the genotype of the bees (Guzmán-Novoa et al., 1999; Rosenkranz, 1999; Martin and Medina, 2004; Invernizzi et al., 2015; Merke, 2016) and by the environment (Meixner et al., 2015; Giacobino et al., 2017). For example, Africanized bees are more resistant to the *Varroa* mite than European bees in many South and Central American regions (Martin and Medina, 2004; Mondragón et al., 2005) and in Puerto Rico (Rivera-Marchand et al., 2012). Differences in *Varroa* infestation rates and mite tolerance were also detected between African subspecies (*A. m. scutellata*, *A. m. capensis*) and hybridized colonies of these two subspecies in South Africa (Mortensen et al., 2016). Meanwhile, recent research in honey bee populations of European origin demonstrated strong genotype—environment interactions and suggested that the presence of a highly significant environmental effect on *Varroa* infestation rates may be stronger than the effect of the bees' genotype (Francis et al., 2013; Büchler et al., 2014). In fact, honey bee colony life histories, driven by environmental conditions, have a significant influence on *Varroa* infestation rates; in consequence, mite infestation thresholds probably vary considerably across localities and regions (Meixner et al., 2015; Giacobino et al., 2017).

Landscape, topography, and climate are relevant environmental drivers that can influence the prevalence of *Varroa* in honey bee colonies (Chemurot et al., 2016; Giacobino et al., 2017; Correia-Oliveira et al., 2018). Landscape composition has been shown to impact on bee health in general, and in *V. destructor* loads in particular (Youngsteadt et al., 2015; Giacobino et al., 2017; Kuchling et al., 2018). However, the relationship between landscape and *Varroa* is not clear, because some studies have reported high prevalence of *Varroa* in colonies where urban land cover dominates (Youngsteadt et al., 2015), while others have described high prevalence of the mite where crops dominate (e.g., Giacobino et al., 2017). Similarly, in relation to topographic and climatic influence, some variables such as altitude, apiaries slope, temperature, and rainfall have been shown to be associated with mite parasitism in honey bees' colonies from tropical and neotropical environments (Chemurot et al., 2016; Giacobino et al., 2017; Correia-Oliveira et al., 2018). Specifically, hot and dry conditions seem to be negatively correlated to the prevalence of the mite at the colony level (Maggi et al., 2016).

The majority of studies examining the role of the environment on the mite load have analyzed parasitism patterns in honey bee colonies, while a large component of mite dynamics is related to bees' movement. In fact, nest drifting in honeybees is believed to be involved in disease transmission between colonies (e.g., Kralj and Fuchs, 2006, 2010; Aubert et al., 2008). This behavior, performed by female and male bees, has been attributed to beekeeping practices and a lack of landmarks (Free, 1958; Southwick and Buchmann, 1995; Nolan and Delaplane, 2017), and has been reported to vary depending on the season, colony characteristics and bee subspecies (Duranville et al., 1991; Pfeiffer and Crailsheim, 1998; Neumann et al., 2000, 2003; Paar et al., 2002; Forfert et al., 2015). At the same time, parasites can influence the drifting behavior of worker bees, and higher occurrence of drifting may occur in infected bees (Bordier et al., 2017). Drone-mediated movement may also play a relevant role in disease transmission in general, and in Varroosis in particular (Nolan and Delaplane, 2017). Compared to the queen or the workers, the biology, dispersion activity, and influence on mites' transmission of honey bee drones are poorly understood.

Male bees represent an important part of the mite-bee' interaction, given that they often drift to other, non-maternal colonies and may spread the mite among them (Neumann et al., 2000; Paar et al., 2002; Abrol, 2012; Gąbka, 2018). In addition, drones and queens from different colonies meet during the spring and summer at mating areas that persist through the years, called Drone Congregation Areas (DCAs) (Zmarlicki and Morse, 1963; Ruttner, 1966). Recently, the presence of *Varroa* in males gathered at DCAs has been reported in the continental US (Mortensen et al., 2018), which suggests that these sites are of utmost importance when studying the dynamics of the parasitization. In Puerto Rico, DCAs have been observed to meet certain landscape characteristics, such as dominance of grassland cover, the presence of surrounding tree curtains, a slope of 20°, and southern orientation (Galindo-Cardona et al., 2012). DCAs' characteristics have been understudied in the southern hemisphere, thus the relationship between environmental characteristics and *Varroa* infestation at these sites remains unclear.

In Argentina, apiculture occurs in four of six eco-climatic regions, and particular honey bees' ecotypes have locally adapted to these regions, with important implications for *Varroa* resistance, bees' productivity, and defensive behavior (Giacobino et al., 2016, 2017; Merke, 2016). For instance, in contrast to what occurs in the temperate regions of the country, honey bee populations exhibit general resistance or tolerance to the mite in the subtropical region, possibly because of the high prevalence of African-derived bees and the warmer temperatures of these regions (Hou et al., 2016; Correia-Oliveira et al., 2018). At the same time, not only climatic or bee genetics, but also agricultural and beekeeping practices, and landscape features, such as urban land cover, differ between regions, and are potentially important determinants of the levels of *Varroa* at apiaries in our territory (Abrol, 2012; Giacobino et al., 2017; Molineri et al., 2018). In addition, the first DCAs have been recently identified in the subtropical region of Argentina (Galindo-Cardona et al., 2017). However, the contribution of these areas to the *Varroa* dispersal and the possible differences in DCA features and *Varroa* parasitism between eco-climatic regions have not been explored yet. Our approach in this study was to address the relative importance of distance to the closest apiary, landscape, topographic, and climatic characteristics on *Varroa* infestation at the DCAs and to characterize genetic diversity in two eco-climatic regions of Argentina (subtropical and temperate). In addition, we intended to environmentally describe DCAs in South America for the first time. Our main hypothesis was that *Varroa* infestation and genetic diversity at the DCAs varied across eco-climatic regions associated with land cover, topographic, and climatic characteristics.

MATERIALS AND METHODS

Study Areas and Material

Commercial and non-commercial apiaries and their respective DCAs were evaluated during 2017 and 2018 in two provinces of Argentina: Tucumán (TU) and Buenos Aires (BA). A total of four apiaries were monitored in TU (Alberdi, Timbó Viejo, Manantial and Horco Molle) and five in BA (two at Luján, two at Castelar, and one in San Antonio de Areco) (**Figure 1**). These provinces belong to different eco-climatic regions of the territory (**Figure 1**). In Tucumán, the climate is subtropical, with precipitations mostly concentrated between October and March, and cold and dry winters (Cabrera and Willink, 1980). The original vegetation of this region (called Yungas forest) includes canopy species of the Fabaceae (e.g., *Parapiptadenia exelsa*), Bignoniaceae (e.g., *Handroanthus impetiginosus),* Lauraceae (e.g., *Ocotea porphyria*), and Malvaceae (e.g., *Ceiba chodatii*) families, among others. For decades, the lowest elevation belt of the Yungas forest has been progressively transformed into agricultural lands, mainly sugarcane and citrus (Gasparri and Grau, 2009) and currently it is a mosaic of croplands bordering the remaining Yungas forests on the eastern slopes of the pre-montane range of the Andes (Morello et al., 2018; Oyarzabal et al., 2018). In Buenos Aires, the climate is temperate, with precipitations occurring between March and June. The original vegetation of the region (Pampa) was a scrub dominated by species of the Zygophyllaceae family and by flowering plants as alfalfas, clovers and trees of the Fabaceae family, such as *Prosopis* sp. and *Geoffroea* sp. (Ferrari et al., 2011). The intensive and persistent agriculture and the rising cattle production in the region has deeply transformed the landscape in the last decades (Morello et al., 2018). The Pampa region concentrates around 65% of the beekeepers in the country. In Buenos Aires there are approximately 1,500,000 hives in contrast to the near 40,000 hives in Tucumán, where the majority of beekeepers are small producers and hardly exceed 30 hives each (Ferrari et al., 2011).

Identification of Drone Congregation Areas

At both eco-regions and for each of the nine reference apiaries, a search of the nearest DCA was performed. DCAs were identified and monitored once in spring and once in summer, for two consecutive years (2017 and 2018) in order to assess their stability through seasons and years. To find a DCA, the route of drones entering and leaving colonies was traced in each reference apiary (Galindo-Cardona et al., 2012, 2017). To this end, a synthetic pheromone (9-hydroxy-2-enoic acid, Phero Tech Inc.) was attached to a helium balloon, which was elevated at a distance of 150 meters from the apiary. This action was repeated multiple times while increasing the distance from the apiary and following the direction of the drones' flights. Searches were carried out intensively during 1 week in each apiary. Each DCA was confirmed where at least 30 drones were observed forming a comet around the bait. At that site, the pheromone was elevated with an unmanned aerial vehicle (UAV) (DJI Phantom 3 STD) at three different points and drone arrival re-checked for a final confirmation.

Evaluation of Varroa infestation

The four apiaries in TU and five in BA were evaluated in spring of 2018 for the *V. destructor* phoretic infestation following De Jong et al. (1982) and Dietemann et al. (2013). Thirty percent of the colonies of each apiary (and up to six honey bee colonies in the smallest apiaries) were sampled randomly. Approximately, three hundred worker bees were collected from each colony and conserved in 96% ethanol. Similarly, at each identified DCA, phoretic *Varroa* infestation was assessed on samples of flying drones, which were attracted using the synthetic pheromone (9-hydroxy-2-enoic acid) attached to an UAV, and collected with a standard entomological net at 1.5–2.5 m above ground. Phoretic *Varroa* infestation was estimated as the number of mites/total number of sampled bees (drones or workers in each case).

Landscape and Topographic Variables

Landscape composition and configuration, and topography were characterized within 400 m-radii buffers around each DCA, given that landscape characteristics have been previously shown to be highly correlated to the presence of DCAs at this distance (Galindo-Cardona et al., 2012). First, a supervised classification of Sentinel-2 satellite images (pixel resolution: 10 m; European Space Agency) was performed using a *random forest* classifier in Google Earth Engine (Gorelick et al., 2017). To do this, winter scenery (June–September 2018) was combined with summer scenery (January–May 2018) with less than 10% of cloudy pixels. Then, land use was categorized within the buffers into: (1) trees (linear forest remnants, groups of trees, and forest), (2) arable land (crops, pastures, and fields with bare soil), and (3) urban use (buildings, paved roads) (**Supplementary Table 1**). In relation to

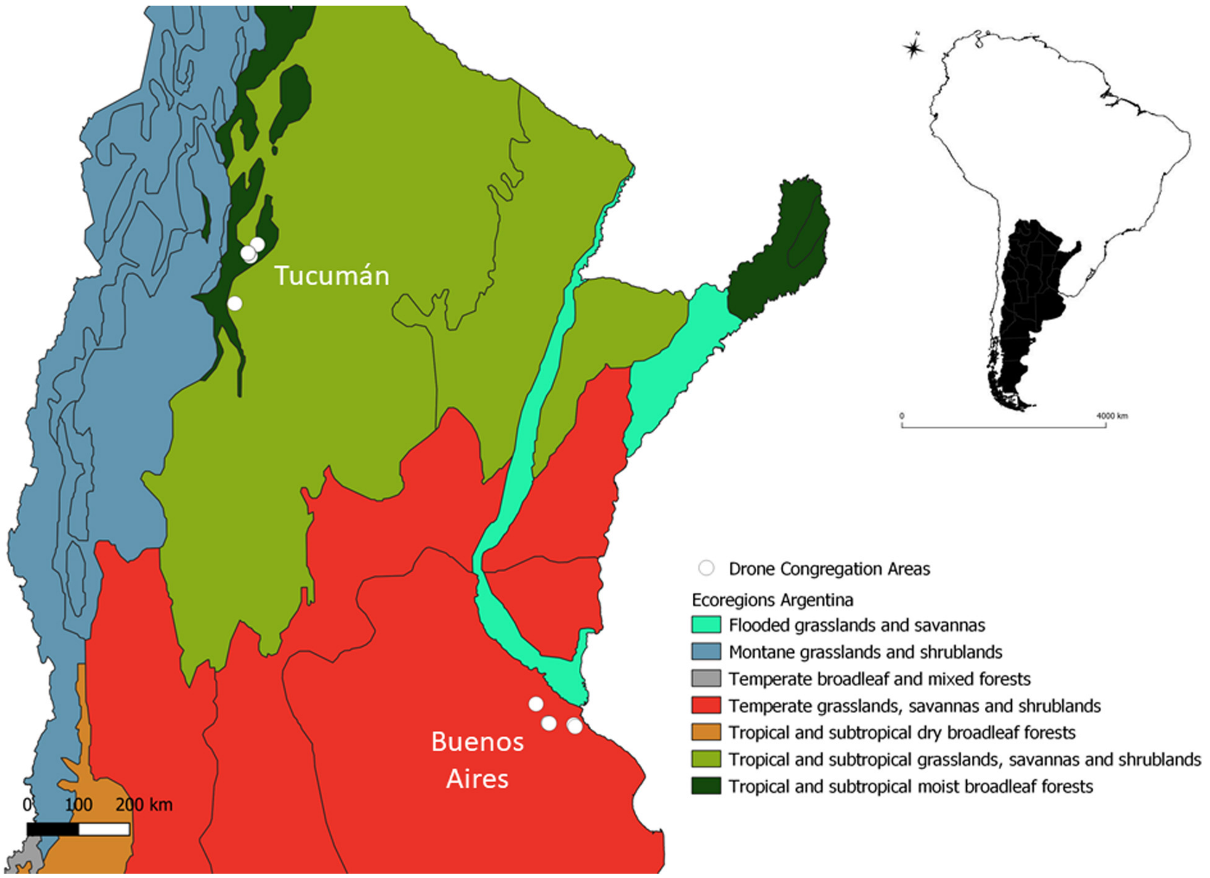

FIGURE 1 | Map of the study area showing the identified Drone Congregation Areas at the two eco-regions in Argentina. Eco-regions were defined according to Olson et al. (2001).

topographic characteristics, a 30 m-resolution Digital Elevation Model from the US Geological Survey (Jarvis et al., 2007) was used to calculate slope and aspect in Google Earth Engine, and roughness in QGIS 2.18 (Geographic Information System [QGIS], 2018) within each buffer (**Supplementary Table 1**).

Climate Variables

Climate data were downloaded from public databases for 2018 for both eco-regions. Data for each identified DCA in Tucumán were downloaded from the Agrometeorology Section of the Obispo Colombres Agro-industrial Experimental Station (EEAOC) website.[1] Data for each identified DCA in Buenos Aires were downloaded from the section of Agrometeorology Management and Information System of the National Institute of Agricultural Technology (INTA)[2] (**Supplementary Table 1**).

Genetic Variability in Apiaries and in Drone Congregation Areas

To identify bees' mt haplotype, newly emerged workers were collected from the central brood frames of colonies of apiaries at Timbó Viejo, Manantial, and Horco Molle (TU), and Luján and Castelar (BA) in spring 2018 (**Figure 1**). One (TU) or two (BA) apiaries per locality and 7–10 colonies per apiary were sampled. Total DNA was extracted from the thorax of one worker per colony following a high-salt protocol (Baruffi et al., 1995). DNA samples of honey bee workers were analyzed using a PCR-RFLP-based method. A fragment of 1,001 bp from the mitochondrial COI-COII region was amplified by PCR using primers and conditions described by Hall and Smith (1991) and Lobo Segura (2000). The amplifications were conducted in a MJ PTC-100 thermal cycler (GMI, Ramsey, MN, United States) and the cycle consisted of an initial denaturation step of 2 min at 94°C followed by 40 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C, followed by a final extension step of 2 min at 72°C. A 10-ll aliquot of each PCR product was digested with *Hin*fI (Promega, Madison, MN, United States) following the manufacturers recommendations. The restriction fragments were separated on 4% (wt/vol) agarose gels, stained with GelRed and photographed under UV light. The haplotypes detected in the restriction analysis using *Hin*fI were assigned as previously described by Agra et al. (2018). A total of 15 and 35 individual worker samples were analyzed for TU and BA, respectively. The same procedure was performed to evaluate the haplotypic lineage

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[1] http://www.eeaoc.org.ar/agromet/index.php

[2] http://siga.inta.gob.ar/#/data

of drones from the identified DCAs; in this case, 20–30 drones from each DCA were analyzed. A total of 78 and 64 individual drone samples were analyzed for TU and BA, respectively. In all cases, except for Castelar Norte and Castelar Sur, we sampled at least twice the number of drones from each DCA than workers from colonies of the surrounding (reference) apiary.

Data Analysis

For statistical analyses, only the proportion of *Varroa* for 2018 was used because data for 2017 were scarce. One site (i.e., Alberdi, TU) could not be visited in 2018, then data for 2017 was used instead. All statistical analyses were conducted in *R* (R Core Team, 2017). The proportion of *Varroa* at the DCAs and at the colonies were arcsin-transformed according to arcsin(sqrt(proportion of infested drones at DCA/colony) for all further analysis. To test for differences in the proportion of *Varroa* between samples (apiaries vs. DCAs) and ecoregions (BA vs. TU) a Two-way ANOVA was performed. To examine the effects of the closest apiaries on the incidence of *Varroa* at the DCAs, two analyses were conducted. First, a linear model (lm) was run on the proportion of infested bees in the colonies as the explanatory variable and the proportion of infested drones at the DCAs as the response variable. A second linear model was performed with the distance of the DCA to the closest apiary as the explanatory variable and the proportion of infested drones at the DCAs as the response variable.

To examine the relationship between *Varroa* infestation at the DCAs and landscape, topographic, and climate variables, several analyses were performed. First, to characterize the DCAs in both eco-regions, a Principal Components Analysis (PCA) on each of the subsets of variables was performed, using landscape, topographic, and climate variables, respectively, in a main matrix. The data were relativized to the maximum value and the PCA was run using a correlation matrix, and a distance—based biplot. A supplier seed = 20 and number of runs = 999 were set in all cases. PCAs were run in PCORD 5.0 (McCune and Mefford, 1999). After performing the PCAs with each data subset (i.e., landscape, topographic, and climate) the eigenvalues of the first two axes were extracted in each case to relate them to the level of *Varroa* at the DCAs. To test these relationships, individual GLMs were run on the proportion of *Varroa* at the DCAs as response variable and both PCA axes were examined as additive factors. In addition, based on the correlations of each variable with the first two PCA axes (**Table 1**), the variables that were most positively and most negatively correlated to each axis were selected (i.e., four variables in total for each data subset) to perform backward stepwise GLM selections. These model selections were performed for the landscape, topographic, and climate datasets separately with the aim of simplifying variable numbers before running the final model. The terms were additive in all cases.

A final process of backward stepwise GLM selections were performed using the proportion of *Varroa* at the DCAs as the response variable; distance of the DCA to the closest colony, and the landscape, topography, and climate variables selected in the previous steps were explanatory variables. The explanatory variables were examined as additive and interacting factors. The GLMs were run using family = gaussian and link = identity. The function *step* in *R* conducts a stepwise selection procedure starting with an arbitrary model, adding or removing a term from the model that most reduces the Akaike's information criterion (AIC) and stops when no step decreases the AIC (Chambers and Hastie, 1993). The AIC identifies the best model taking into account both the sample size and the number of predictors (Quinn and Keough, 2002); models with the lowest AIC are the best fit, more parsimonious models.

To analyze possible differences in haplotype frequencies between eco-regions (TU vs. BA) and between apiaries and DCAs for each region, Chi-square tests were performed. Spearman's rank order correlations between the percentage of *Varroa* and the percentage of Africanized haplotypes were performed separately for DCAs and apiaries.

TABLE 1 | Main correlates between landscape, topographic, and climatic metrics analyzed using Principal Components Analysis and the first two PCA axes in each analysis.

Variable	Correlates with Axis 1	Variable	Correlates with Axis 2
Landscape			
Number of patches arable	−0.895	Landscape division trees	−0.933
Like adjacencies urban	−0.855	Splitting Index trees	−0.677
Edge density urban	−0.849	Landscape proportion arable	−0.671
Mean patch area arable	0.795	Like adjacencies trees	0.973
Overall core area arable	0.822	Largest Patch Index trees	0.974
Like adjacencies arable	0.942	Greatest patch area trees	0.979
Topography			
Aspect northing SD	−0.953	Aspect easting SD	−0.777
Aspect northing mean	−0.866	Roughness SD	−0.578
Aspect easting SD	−0.580	Slope SD	−0.413
Roughness mean	0.964	Elevation SD	−0.057
Slope mean	0.968	Elevation mean	0.075
Elevation SD	0.978	Aspect easting mean	0.817
Climate			
Wind week	−0.953	Humidity before	−0.648
Wind before	−0.943	Solar radiation	−0.338
Pressure before	−0.935	Temperature week	−0.296
Precipitation week	0.764	Precipitation week	0.443
Humidity week	0.891	Temperature before	0.577
Temperature week	0.944	Precipitation before	0.690

In each data subset the three most positive and the three most negative correlates are shown for each axis, according to the r coefficient output in the PCA.

RESULTS

We found a total of 11 DCAs, five in 2017 and six more in 2018 (**Supplementary Table 1**). All DCAs found in 2017 persisted in 2018. The maximum distance between each DCA and the closest apiary was 1,000 m and the average distance between DCAs and apiaries was 500 m. Specifically for the locality of Manantial (TU), two DCAs were detected and since these were separated by 100 m, only the one that was confirmed in both years was included in the analyses. In the locality of Horco Molle (TU) three DCAs, separated by 1,000 m, were identified and all were included in the analyses. For the rest of the localities, only one DCA was identified per reference apiary.

Varroa Infestation at Apiaries and Drone Congregation Areas

In 2018, the mean proportion of *Varroa* was 0.0028 (SD: 0.0046) at the apiaries and 0.0168 (SD: 0.0227) at the DCAs (**Supplementary Table 1**). *Varroa* infestation was not significantly different between apiaries and DCAs ($F = 0.6179$, df = 1, $p = 0.442$) or between BA and TU ($F = 0.112$, df = 1, $p = 0.742$) (**Figure 2**). The proportion of *Varroa* at the DCAs was not linearly correlated to the proportion of *Varroa* at the apiaries (Adj. $R^2 = -0.1186$, $p = 0.8357$; **Figure 3A**), but it was positively correlated to the distance to the apiaries (Adj. $R^2 = 0.3718$, $p = 0.02734$; **Figure 3B**). Specifically, the proportion of *Varroa* at the DCAs varied between 0 and 0.02 up to 800 m away from the apiaries, and increased to more than 0.06 at higher distances.

Environmental Variables and Varroa Infestation at Drone Congregation Areas

The dominant land use around all DCAs was arable lands, which varied between 28 and 88% and showed greater connectivity and more regular-shaped patches than natural vegetation and urban covers. The multivariate analysis showed that PCA Axis 1 explained 37.25% and Axis 2 explained 30.19% of the variation in the landscape features characterizing the DCAs, respectively (**Figure 4A**). Cumulatively, Axes 1–4 explained 86.72% of this variation. DCAs in TU showed landscape characteristics that separated them from DCAs in BA, in general. Axis 1 separated sites surrounded by continuous arable land (right part of the axis) from sites surrounded by urban land cover (left part of Axis 1) (**Table 1** and **Figure 4A**). DCAs surrounded by continuous arable land showed high values of the following metrics calculated from the arable land cover: Mean Patch Area, Overall Core Area, and Like Adjacencies (i.e., contiguity). In contrast, DCAs surrounded by urban land cover showed high values of Number of arable land patches (i.e., the arable cover was more fragmented), and high values of Like Adjacencies and Edge Density of the urban cover. Axis 2, which explained as much variation in the data as Axis 1, separated DCAs located at TU from those located at BA more clearly than Axis 1. The main variable differentiating DCAs at both ecoregions was the configuration of the natural vegetation land cover. DCAs at the upper part of the ordination (i.e., high values in Axis 2) had high values of Greatest Patch Area, Largest Patch Index, and Like Adjacencies of the natural vegetation land cover. On the other hand, DCAs located at the lower part of the ordination, showed high values of Landscape Proportion of the arable cover and high values of Landscape Division and Splitting Index of the natural vegetation cover.

In relation to topography, Axis 1 explained 70.6% and Axis 2 explained 19.0% of the variation in the PCA run on the DCAs. Cumulatively, Axes 1–4 explained 96.62% of this variation. DCAs in TU were more variable in terms of topographic characteristics than DCAs in BA. Axis 1, which explained most of the variation in the ordination, separated DCAs located at a more variable

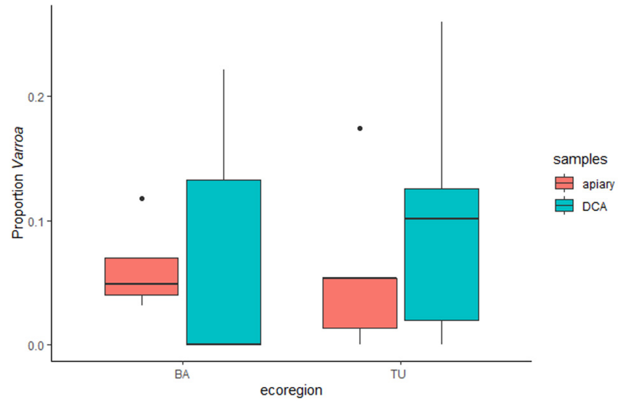

FIGURE 2 | Box-and-whisker plot of the arcsine-transformed proportion of *Varroa* in samples taken from apiaries and from Drone Congregation Areas at the two ecoregions, Buenos Aires (BA) and Tucumán (TU). Each box shows the lower and upper quartiles, the black line within the box is the median, and the error bars are the minimum and maximum values, respectively.

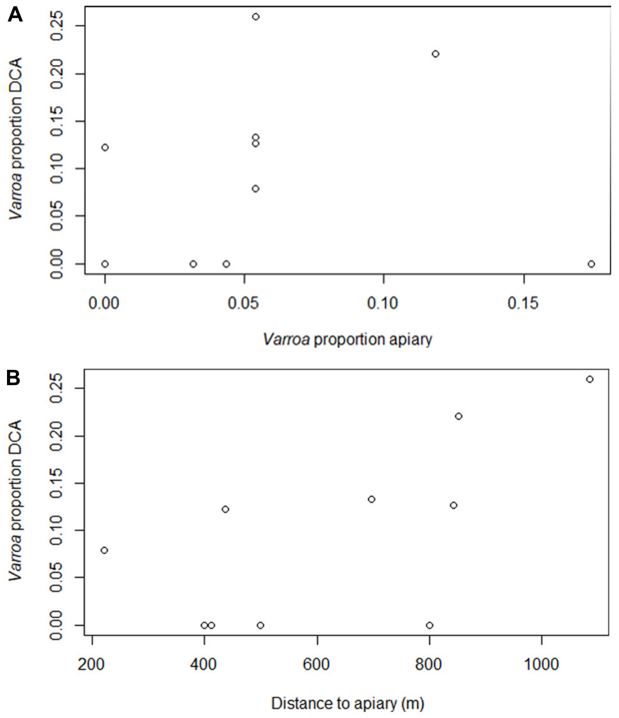

FIGURE 3 | Proportion of *Varroa* infestation at the DCAs in relation to **(A)** proportion of *Varroa* infestation at the closest apiary, and **(B)** distance to the closest apiary. Data from the two eco-regions of Argentina were included in the analysis. Proportion of *Varroa* infestation was arcsine-square root-transformed.

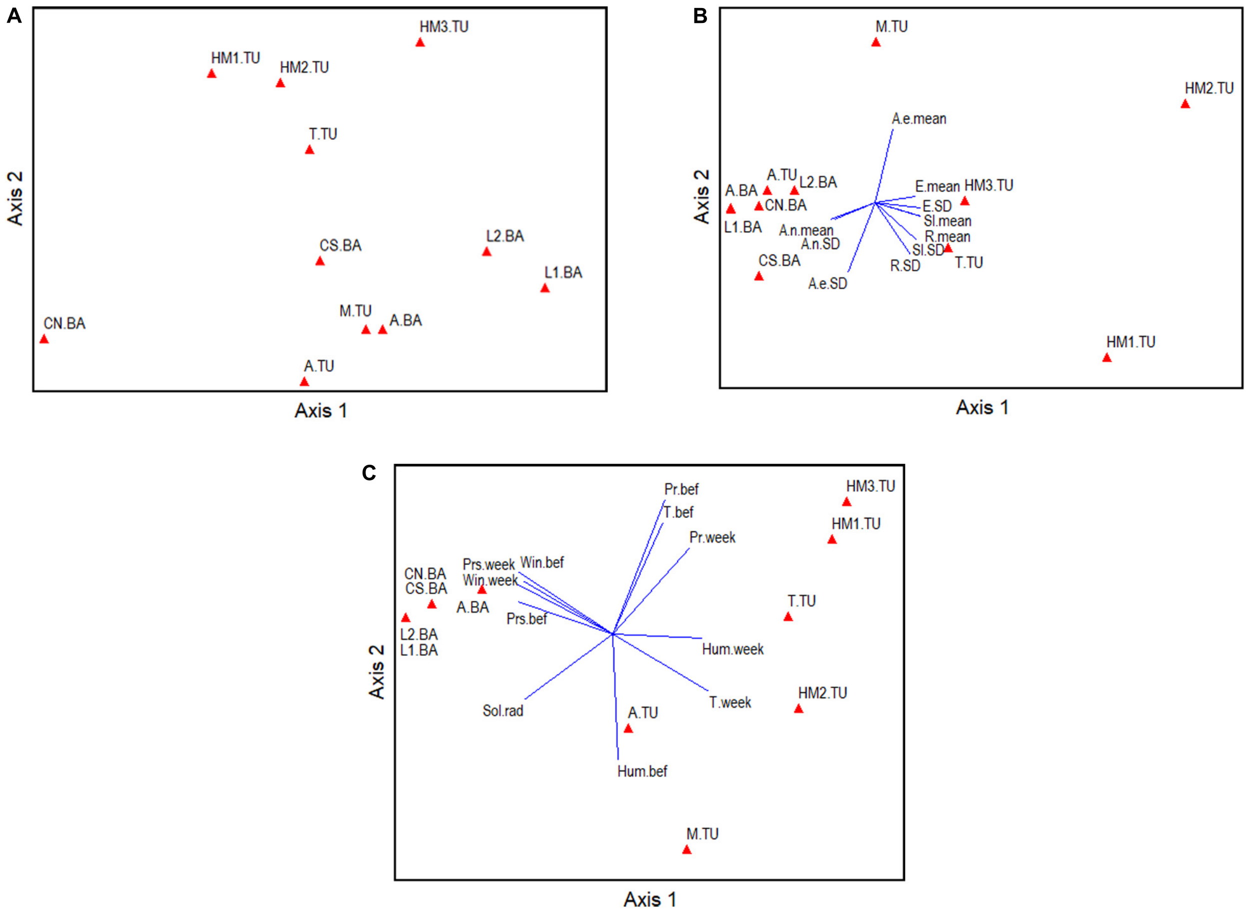

FIGURE 4 | Principal Components Analyses of the Drone Congregation Areas located in two eco-regions in Argentina. PCAs on **(A)** landscape variables, **(B)** topographic variables, and **(C)** climate variables using a correlation matrix in all cases (**Supplementary Table 1** for coding).

elevation above sea level (right part of the axis) from DCAs at slopes facing to the north (left part of Axis 1) (**Table 1** and **Figure 4B**). The DCAs at the right part of the axis were mostly in TU where DCAs were located at a maximum of 688 m.a.s.l. In addition to showing high values of Elevation Standard Deviation (e.g., 17.45), they showed high values of Mean Slope (5.6°) and Mean Roughness (**Supplementary Table 1**). Roughness derives from elevation and represents the physical morphology of the sites where DCAs were located. In contrast, at the left part of the axis, DCAs were mostly from BA, which, in addition to low elevation above sea level (from 8 m.a.s.l.), showed high values of Aspect northing Standard Deviation and Aspect northing mean, followed by Aspect easting Standard Deviation. Axis 2 did not clearly separate DCAs at the ecoregion level. The main variable differentiating DCAs in this axis was Aspect easting mean, which was positively related to Axis 2. To a lesser degree, Elevation mean and Standard Deviation characterized DCAs at the upper part of the ordination. In contrast, at the lower part of the ordination, DCAs showed high values of Aspect easting Standard Deviation, Roughness Standard Deviation, and Slope Standard Deviation.

The DCAs were located in sites under different climatic characteristics (**Figure 4C**). Axis 1 in the PCA explained 63.76% and Axis 2 explained 17.25% of the variation in climate variables. Cumulatively, Axes 1–4 explained 96.27% of this variation. In comparison with the landscape and topographic ordinations, PCA runs on climate variables more clearly separated DCAs in TU from DCAs in BA. Axis 1 separated DCAs located at sites with high mean temperature in TU (right part of the axis) from DCAs at sites with high values of wind velocity in BA (left part of Axis 1) (**Table 1** and **Figure 4C**). In addition, DCAs in TU showed high values of relative humidity and mean precipitation while DCAs in BA showed high values of both wind velocity and atmospheric pressure the week before sampling. Axis 2 was positively correlated to both, mean precipitation and mean temperature the week before sampling, and to mean precipitation on the week of sampling (i.e., DCAs in the upper part of the ordination) (**Table 1** and **Figure 4C**). At the same time, Axis 2 was negatively correlated to relative humidity 1 week before sampling, solar radiation, and mean temperature on the week of sampling (i.e., DCAs in the lower part of the ordination).

The proportion of *Varroa* at the DCAs was not related to the PCA' eigenvalues in any of the three data subsets (i.e., landscape, topography, climate) (**Table 2**). When landscape, topographic, and climate variables were tested in separate GLMs (i.e., one

GLM for each data subset) as additive terms, the following were significantly correlated to the proportion of *Varroa* at the DCAs: Distance of DCA to closest apiary, Landscape Division of the natural vegetation cover, mean precipitation 1 week before sampling, and relative humidity 1 week before sampling. These variables were considered in the final backward stepwise GLM selections where the full model contained the simple terms, quadratic terms, and interactions. The best resulting model was: Proportion *Varroa* at DCA = Distance of DCA to closest apiary + Humidity 1 week before + (Precipitation 1 week before)^2 + Distance of DCA to closest apiary * Humidity 1 week before * (Precipitation 1 week before)^2 (Residual deviance: 0.0017315 on 3 degrees of freedom, AIC: −47.106) (**Table 3** and **Figure 5**).

TABLE 2 | Coefficients resulting from Generalized Linear Models (GLM) where the proportion of drones infested with *Varroa* at the Drone Congregation Areas was the response variable.

	Estimate	Std. error	*t*-value	Pr(>|t|)	AIC
Landscape					−17.571
(Intercept)	0.085711	0.026756	3.203	**0.0125**	
PCA Axis 1	−0.008737	0.005911	−1.478	0.1776	
PCA Axis 2	0.007567	0.006566	1.153	0.2824	
Topography					−14.597
(Intercept)	0.085711	0.030630	2.798	**0.0233**	
PCA Axis 1	0.009171	0.011528	0.796	0.4492	
PCA Axis 2	0.008671	0.022228	0.390	0.7066	
Climate					−14.789
(Intercept)	0.085711	0.030363	2.823	**0.0224**	
PCA Axis 1	0.009179	0.011465	0.801	0.4465	
PCA Axis 2	0.012055	0.022040	0.547	0.5993	

The GLMs were performed separately using as explanatory variables the Principal Component Analysis' eigenvalues for Axis 1 and Axis 2 in the landscape, topographic, and climate data subsets. The Akaike Criterion (AIC) is shown for each GLM. The general formula for the GLMs was: Varroa proportion = PCA Axis 1 + PCA Axis 1, family = gaussian. Significant P-values are shown in bold.

TABLE 3 | Coefficients resulting from backward stepwise deletions using the proportion of drones infested with *Varroa* at the Drone Congregation Areas (DCAs) as the response variable.

	Estimate	Std. error	*t*-value	Pr(>|t|)
Intercept	−1.384e + 00	4.405e-01	−3.141	0.0516
Dist. apiary	1.992e-03	8.268e-04	2.410	0.0950
Hum. before	1.926e-02	5.916e-03	3.256	**0.0473**
(Prec. before)^2	1.170e-02	2.426e-03	4.823	**0.0170**
Dist. apiary: Hum. before	−2.767e-05	1.134e-05	−2.440	0.0925
Dist. apiary: (Prec. before)^2	−2.628e-05	5.734e-06	−4.584	**0.0195**
Hum. before: (Prec. before)^2	−1.625e-04	3.375e-05	−4.814	**0.0171**
Dist. apiary: Hum. before: (Prec. before)^2	3.656e-07	7.974e-08	4.585	**0.0195**

The explanatory variables resulting from the Principal Component Analyses were distance to the apiary (Dist. apiary), and the landscape, topographic, and climate variables of the DCAs. Hum. Before, percent humidity 1 week before Varroa estimation; (Prec. before)^2: square of mean precipitation 1 week before Varroa estimation. Significant P-values are shown in bold.

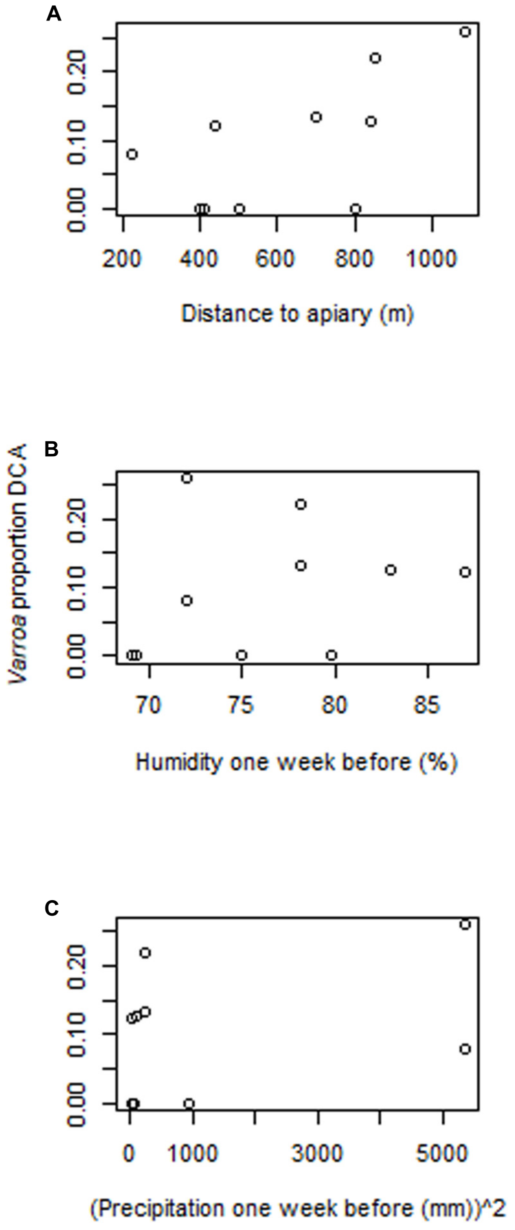

FIGURE 5 | Relationships between the proportion of *Varroa* in males at the Drone Congregation Areas and the environmental variables identified by the automatic Generalized Linear Model backward selection in *R*. **(A)** Distance from the DCA to the closest apiary, **(B)** humidity 1 week before *Varroa* estimation, and **(C)** precipitation 1 week before *Varroa* estimation. Proportion of *Varroa* infestation was arcsine-square root-transformed.

Genetic Variability

The analysis of workers from honey bee colonies and drones from DCAs revealed the presence of four mitochondrial haplotypes (**Figure 6**). Two haplotypes corresponded to evolutionary branch A (A1, A4), and two haplotypes corresponded to branch C (C1 and C2J). The African haplotypes A1 and A4 have been observed at a high frequency in African subspecies *A. mellifera intermissa* and *A. m. scutellata*, respectively, while the C1

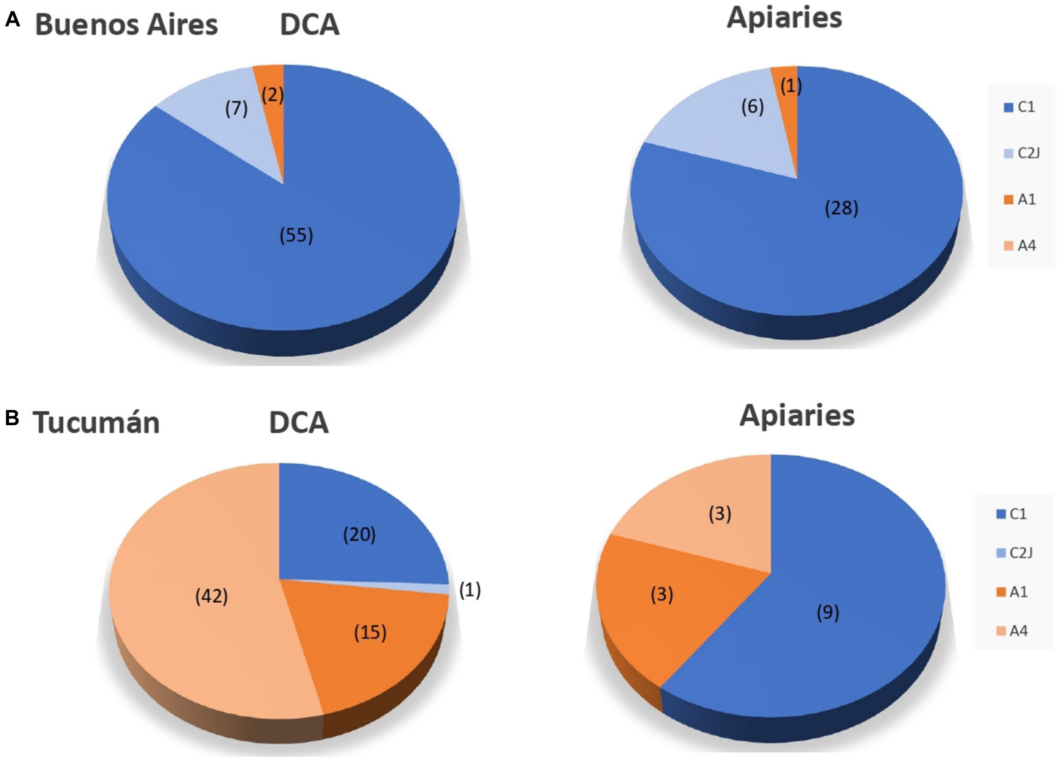

FIGURE 6 | Percentage of mitochondrial lineages of drones at the Drone Congregation Areas and workers at the nearest apiaries for **(A)** Buenos Aires and **(B)** Tucumán provinces. African-derived (A1, A4) and European-derived mitochondrial lineages (C1, C2j) are shown in orange and blue, respectively. Number of analyzed bees are shown between brackets.

haplotype corresponds to the East-European evolutionary lineage that includes the Italian honey bee *A. m. ligustica* and also the Carniolan honey bee (*A. m. carnica)*. The haplotype diversity was similar between TU and BA, with three (C1, C2J, A1) and four (C1, C2J, A1, A4) haplotypes identified, respectively (**Figure 6**). However, the relative frequency of the identified haplotypes differed between eco-regions, and also between DCAs and their surrounding apiaries (specifically in the case of TU, subtropical region) (**Figure 6**). For the temperate region (BA), European haplotypes were found in a high percentage at DCAs (96.9%) and apiaries (97.1%), with no differences between them ($\chi^2 = 0.01$, $P > 0.05$). Haplotype C1 was the most prevalent of C lineage (found in 97% of the samples) and haplotype C2J was found in low frequency (found in 3% of the samples). Respect to the African mt lineage, only 3% of the analyzed samples from Buenos Aires were identified as haplotype A1, while the A4 haplotype was not found (**Figure 6**). Conversely, in the subtropical region, a higher mean percentages of A haplotypes was detected in both DCAs (73.1%) and apiaries (40.0%) when compared to temperate region ($\chi^2 = 70.83$, $P < 0.0001$ for DCAs; $\chi^2 = 12.03$, $P = 0.001$ for apiaries). Significant differences in the mean percentage of A lineage were identified between DCAs and the nearest apiaries for this region ($\chi^2 = 6.3$, $P < 0.05$). In these cases, two haplotypes (A1 and A4) were detected, being A4 the most prevalent in drones from the DCAs.

When distribution of haplotypes was analyzed within each region (BA or TU), a similar pattern was detected among the three localities from BA (Additional File S2). Conversely, the distribution of haplotypes was dissimilar among localities of Tucumán (Additional File S3). Specifically, DCAs near to non-commercial apiaries (Manantial and Horco Molle) presented a high representation of African haplotypes (85 and 100%, respectively), while the DCA near to a commercial apiary (Timbó) only exhibited 18.2% of A haplotypes (Additional File S3). The frequencies of haplotypes A were different between the two types of DCAs (Timbó vs. Manantial: $\chi^2 = 23.9$, $P < 0.001$; Timbó vs. Horco Molle: $\chi^2 = 37.5$, $P < 0.001$; Horco Molle vs. Manantial: $\chi^2 = 3.7$, $P > 0.05$). Finally, no significant correlation was detected between the percentage of *Varroa* and the percentage of A haplotypes in DCAs or apiaries.

DISCUSSION

In this study, we presented an integral description of main characteristics of the landscape, topography, climate, *Varroa* infestation status, and genetic variability of the Drone Congregation Areas and identified environmental variables associated to mite infestation in two eco-climatic regions from Argentina.

Environmental characteristics of the DCAs within a 400 m-radius buffer in the subtropical (TU) and temperate (BA) regions in Argentina were highly variable. With respect to landscape, DCAs were surrounded by all three types of matrices (i.e., natural vegetation, arable lands, and urban), and the presence and contiguity of natural vegetation was the main difference between ecoregions. In relation to topography, DCAs were located at elevations as low as 8 m.a.s.l. and up to 688 m.a.s.l. Topographic characteristics were more distinct among ecoregions than landscape characteristics describing DCAs and included elevation variability (higher in TU) and aspect in the north-south direction (northern aspect in BA). Lastly, DCAs occurred under highly variable climatic conditions, from windy (5.73 km/h) and cooler (18°C) locations in BA, to less windy (1.3 km/h) and warmer (22.3°C) locations in TU. DCAs in both ecoregions were better differentiated by climatic conditions than by topography and landscape.

Most previous observations of DCA environmental descriptors have been conducted in the northern hemisphere (Europe: Baudry et al., 1998; Koeniger G. et al., 2005; Koeniger N. et al., 2005; Kraus et al., 2005; and Asia: Punchihewa et al., 1990; Wattanachaiyingcharoen et al., 2008), in South Africa (Muerrle et al., 2007; Jaffé et al., 2009; Yañez et al., 2012), and in Australia (Arundel et al., 2013). Similarly to previous evidence, DCAs in our South American sites were located in vegetation clearings, where contrasting vertical relieves, such as a forest-pasture boundary, lines of trees, or buildings seemed to be important landmarks (Ruttner, 1966; Strang, 1970; Loper et al., 1992; Quezada-Euan and Jesus May-Itza, 2006; Muerrle et al., 2007). Other similarities with previous works are that DCAs in our sites were variable in terms of topography (Koeniger and Koeniger, 2000) but located in slopes < 20% (Galindo-Cardona et al., 2012), and they were variable in terms of climatic conditions (Strang, 1970). On the other hand, our sites in Argentina showed some differences with previous studies. First, it has been shown that urban land cover of less than 10% improved DCA occurrence in the subtropical Puerto Rico (Galindo-Cardona et al., 2012), while some of our DCAs, including temperate and subtropical sites, were surrounded by more than 50% urban cover. Thus, the role of urban cover in the formation of DCAs needs to be further explored. In addition, DCAs in Puerto Rico had slope orientations (i.e., aspect) more concentrated toward the South than locations where no DCAs were encountered, and this pattern was proposed to be a response to wind direction, sunlight, and magnetism (Galindo-Cardona et al., 2012). Our data showed that half of the DCAs (i.e., those in BA) had a northern aspect. This may be related to protection from wind currents, which occur from the southeast in this region of Argentina. This implies that wind direction may be an environmental character to address when describing DCAs in other regions of the world. In contrast, solar radiation, as a surrogate of sunlight, was not a main environmental variable differentiating DCAs among ecoregions in our study. Further assessments need to incorporate environmental characteristics of non-DCAs vs. DCAs in order to build statistical models for DCAs prediction.

Extremely low levels of *Varroa* infestation were detected in the present study, with no significant differences between apiaries and DCAs. However, a trend toward higher loads in DCAs suggests that a preference of the mite for drones in comparison to workers may be operating as previously suggested for immature stages (Boot et al., 1995). A similar pattern has been observed for the loads of Deformed Wing Virus of drones in South Africa, where drones demonstrated to be able to reach DCAs in spite of the infection (Yañez et al., 2012). Even though the mite loads were not significantly different between ecoregions in Argentina, the maximum values of *Varroa* infestation were reached in the subtropical TU. This apparent difference would be in line with the current beekeeping practices applied in the country (Giacobino et al., 2016), where acaricide applications are more frequent in the temperate BA due to the susceptibility of honey bee populations of European origin to the mite infestation. In TU, as in the rest of the northern region of Argentina, the control of *Varroa* is scarce due to low density of hives, limited economic resources for apiculture, and high prevalence of Africanized honey bees. Giacobino et al. (2017) found that the environment, measured using climate and landscape variables, was more important than management as a driver of *V. destructor* infestation in the territory in autumn. In our study, no statistical differences among ecoregions were detected in spring, suggesting that seasonal differences on the relative weights of *Varroa* drivers may exist. It is worth characterizing the *Varroa* loads in summer, where the mite's populations increase in colonies and hence probably at the DCAs. The low levels of infestation detected in spring could be preventing the detection of differences between apiaries and DCA and also between ecoregions. Further characterization of *Varroa* levels at both apiaries and DCAs for all three seasons of bee activity (i.e., spring, summer, autumn) would shed light on the general dynamics of parasitization since a great variation occurs between years (Harris et al., 2003).

No correlation between *Varroa* infestation at the apiaries and at the DCAs was observed, but *Varroa* infestation at the DCAs was positively correlated to the distance to the closest managed apiary. Given that drones prefer closer than farther DCAs (Koeniger N. et al., 2005), we speculate that those DCAs that were located at higher distances from the closest managed apiary received a relatively higher proportion of mite-infested drones from feral colonies. In this sense, both the application of acaricides in the apiaries and the input of feral colonies to DCAs would have an effect in the mite infestation level at these sites, independently of the ecoregion. Taking into account our results, we suggest that the assessment of mite infestation (or other pathogens) in DCAs could be a diagnostic measure of the health of honey bees (both managed and feral) in a particular area. This measure would improve *Varroa* monitoring strategies by reducing the time and economic costs of evaluating colonies of all the apiaries of a particular area. In relation to other environmental drivers of parasitism, such as climate, additional research is needed to confirm associations, given the low infestation rates detected in our study.

Here, we provided a first description of the genetic variability found in DCAs and their surroundings apiaries from Argentina by identifying the mytotype origin of drones and workers honey bees. We detected the presence of both Africanized and European mitochondrial lineages but at different relative frequencies between the two analyzed regions. According to the previous results obtained by Abrahamovich et al. (2007), we found a high frequency of European haplotypes in the apiaries from the temperate region. This is not surprising as Argentinian beekeepers have imported queens from Europe since the beginning of beekeeping activities in the country. Moreover, in order to conserve the high productivity and gentle character of these bees, local beekeepers have selected these populations based on the yellowish colour of the abdomen of the Italian queens (Abrahamovich et al., 2007). As we expected, African haplotypes were detected at higher frequencies in apiaries from the subtropical region compared to the temperate region. These results are in line with pioneer studies from Sheppard et al. (1991) and Whitfield et al. (2006) and more recent work from Agra et al. (2018), who found a latitudinal cline from north to south for the level of hybridization between Africanized and European honey bees. In addition, these results agree with those from Diniz et al. (2003) for honey bee populations from southern Brazil and Uruguay.

The pattern of haplotypic distribution found between regions for workers (apiaries) was consistently similar for drones (DCAs). However, the DCAs appeared to be more diverse in terms of the quantity and frequencies of the detected haplotypes. These results agree with previous works in which DCAs concentrate drones from several apiaries and feral colonies (Baudry et al., 1998; Collet et al., 2009). Related to the comparisons between DCA and apiaries, we observed a similar pattern of genetic diversity for the temperate region, where the beekeeping is intensive and principally based on European-derived honey bees. Conversely, in the subtropical region, a different pattern of haplotypic frequencies was detected between DCAs and apiaries, with DCAs showing a high frequency of African haplotypes and apiaries showing a higher representation of European haplotypes. These results may suggest that in the subtropical region, despite the beekeepers also using European honey bee stocks in their apiaries, a great contribution of African haplotypes (probably from feral colonies or swarms) to DCAs occurs. In addition, a high natural queen turnover at commercial apiaries occurs in this region. These results probably reflect that DCAs of northern regions of Argentina are more dynamic systems and maintain high genetic diversity compared to southern DCAs, in line with what Jaffé et al. (2009) previously described for African (*A. mellifera scutellata*) DCAs. These authors found a high turnover of wild colonies that resulted in a temporal genetic differentiation and an effective population size of the DCA. In our case, when non-commercial apiaries (Manantial, Horco Molle) from the subtropical region were analyzed, this pattern was particularly evident. Moreover, this result is in line with previous findings by Mortensen and Ellis (2016) who found that DCAs distant to managed European honey bee apiaries had significantly more African matriline drones than did DCAs located close to managed European honey bee apiaries.

Our results are also consistent with observations made by Collet et al. (2009), who assessed the genetic structure of DCAs and commercial European-derived and Africanized apiaries in southern Brazil. These authors, by employing microsatellite loci, found high genetic similarity between colonies of commercial apiaries and DCAs formed nearby, and differences in genetic structure of Africanized DCAs when compared to the European. It is worth to mention that in our study European drones in DCAs near the apiary of Manantial (TU) were underrepresented, while all colonies were of European origin. If drones actually chose the nearest DCA (Koeniger N. et al., 2005), our results could evidence (i) a differential drone contribution of European and Africanized colonies to DCAs (as was observed in Collet et al., 2009), probably with the former drones being displaced by Africanized ones or (ii) colonies from commercial apiaries are underrepresented in the nearest DCAs because of a high density of non-commercial colonies in the area. Given the mentioned trend toward higher *Varroa* loads in DCAs vs. apiaries, specifically in the subtropical region, further studies need to determine if the relatively higher *Varroa* parasitism detected at DCA represents an indirect evidence of the tolerance to the mite in these Africanized populations. Understanding these mechanisms will add light into current debate about the vulnerability of honey bee races to different diseases.

Previous evidence (e.g., Collet et al., 2009; Mortensen and Ellis, 2016) jointly with our present results suggest that the proportion of drones of a particular genetic origin in DCAs can be influenced by drone flooding. This technique could have a strong influence in selective breeding programs aimed at influencing mating to partially control the paternal contribution in breeding populations of honey bees. In fact, Guzman-Novoa and Page (1999) conducted a long-term selective breeding program in an open honey bee population of more than 3,000 European-derived colonies located in an Africanized area in Mexico, and found that Africanization was diluted within interference from instrumental insemination. Studying these areas where European-derived and Africanized colonies coexist represents an opportunity for checking how these honey bee populations cooperate in forming a DCA, the dynamics of these sites, and the actions that must be considered for specific breeding strategies.

CONCLUSION

Our results provide valuable information on the dynamics of *Varroa* parasitism in apiaries and DCAs, and highlight the role of drones in mite dispersion and genetic diversity of new colonies. We demonstrated that distance to the closest apiaries, relative humidity, and precipitation the previous week need to be taken into account when examining *Varroa* infestation at the DCAs. Further studies need to incorporate DCAs and non-DCAs' environmental characteristics in order to model DCA occurrence and *Varroa* loads with more accuracy. Our study also provides a new step toward the study of the genetic variability of DCAs in the Southern hemisphere where European and African-derived honey bee populations coexist. The study of DCAs in general emerges as a tool for investigating not only honey bee reproduction and breeding, but also the impact of the environment on bee epidemiology.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

AG-C, AS, and AM-G: methodology and analysis, conceptualization, validation and visualization, resources and project administration, supervision and writing—original draft preparation. AG-C, AS, RR, KE, MG, NL, MA, IM, ML, LL, TG, and AM-G: investigation and writing—review and editing. AS and AM-G: software and data curation. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2020.590345/full#supplementary-material

Supplementary Figure 1 | Percentage of mitochondrial lineages of drones at the Drone Congregation Areas and workers at the nearest apiaries for the sites at Buenos Aires. African-derived (A1, A4) and European-derived lineages (C1, C2j) are shown in orange and blue, respectively. Numbers of analyzed bees are shown between brackets.

Supplementary Figure 2 | Percentage of mitochondrial lineages of drones at the Drone Congregation Areas and workers at the nearest apiaries for the sites at Tucumán. African-derived (A1, A4) and European-derived lineages (C1, C2j) are shown in orange and blue, respectively. Numbers of analyzed bees are shown between brackets.

REFERENCES

Abrahamovich, A. H., Atela, O., De la Rúa, P., and Galián, J. (2007). Assessment of the mitochondrial origin of honey bees from Argentina. *J. Apic. Res.* 46, 191–194. doi: 10.1080/00218839.2007.11101391

Abrol, D. P. (ed.). (2012). "The problem of diseases in bees," in *Pollination Biology*, (Dordrecht: Springer), 603–633. doi: 10.1007/978-94-007-1942-2_18

Agra, M. N., Conte, C. A., Corva, P. M., Cladera, J. L., Lanzavecchia, S. B., and Palacio, M. A. (2018). Molecular characterization of *Apis mellifera* colonies from Argentina: genotypic admixture associated with ecoclimatic regions and apicultural activities. *Entomol. Exp. Appl.* 166, 724–738. doi: 10.1111/eea.12719

Arundel, J., Oldroyd, B. P., and Winter, S. (2013). Modelling estimates of honey bee (*Apis* spp.) colony density from drones. *Ecol. Modell.* 267, 1–10. doi: 10.1016/j.ecolmodel.2013.07.008

Aubert, M., Ball, B., Fries, I., Moritz, R., Milani, N., and Bernardinelli, I. (2008). *Virology and the Honey Bee*. Luxembourg: Publications Office of the European Union, 462. Available online at: http://www.eurosfaire.prd.fr/7pc/doc/1262083725_virology_and_the_honey_bee.pdf

Baruffi, L., Damiani, G., Guglielmino, C. R., Bandi, C., Malacrida, A. R., and Gasperi, G. (1995). Polymorphism within and between populations of *Ceratitis capitata*: comparison between RAPD and multilocus enzyme electrophoresis data. *Heredity* 74, 425–437. doi: 10.1038/hdy.1995.60

Baudry, E., Solignac, M., Garnery, L., Gries, M., Cornuet, J. M., and Koeniger, N. (1998). Relatedness among honeybees (*Apis mellifera*) of a drone congregation. *Proc. R. Soc. B Biol. Sci.* 265, 2009–2014. doi: 10.1098/rspb.1998.0533

Boot, W. J., Schoenmaker, J., Calis, J. N. M., and Beetsma, J. (1995). Invasion of *Varroa jacobsoni* into drone brood cells of the honey bee, *Apis mellifera*. *Apidologie* 26, 109–118. doi: 10.1051/apido:19950204

Bordier, C., Pioz, M., Crauser, D., Le Conte, Y., and Alaux, C. (2017). Should I stay or should I go: honeybee drifting behaviour as a function of parasitism. *Apidologie* 48, 286–297. doi: 10.1007/s13592-016-0475-1

Büchler, R., Costa, C., Hatjina, F., Andonov, S., Meixner, M. D., Le Conte, Y., et al. (2014). The influence of genetic origin and its interaction with environmental effects on the survival of *Apis mellifera* L. Colonies in Europe. *J. Apic. Res.* 53, 205–214. doi: 10.3896/IBRA.1.53.2.03

Cabrera, A. L., and Willink, A. (1980). *Biogeografía de América Latina*. Zaragoza: Sociedad Entomológica Aragonesa.

Chambers, J. M., and Hastie, T. J. (1993). *Statistical Models in S*. London: Chapman & Hall.

Chemurot, M., Akol, A. M., Masembe, C., de Smet, L., Descamps, T., and de Graaf, D. C. (2016). Factors influencing the prevalence and infestation levels of *Varroa destructor* in honeybee colonies in two highland agro-ecological zones of Uganda. *Exp. Appl. Acarol.* 68, 497–508. doi: 10.1007/s10493-016-0013-x

Collet, T., Cristino, A. S., Quiroga, C. F. P., Soares, A. E. E., and Del Lama, M. A. (2009). Genetic structure of drone congregation areas of Africanized honeybees in southern Brazil. *Genet. Mol. Biol.* 32, 857–863. doi: 10.1590/s1415-47572009005000083

Correia-Oliveira, M. E., Mercês, C. D. C., Mendes, R. B., Neves, V. S. L., Silva, F. L., and Carvalho, C. A. L. (2018). Can the environment influence varroosis infestation in Africanized honey bees in a neotropical region? *Fla. Entomol.* 101, 464–469. doi: 10.1653/024.101.0304

De Jong, D., De Jong, P. H., and Gonçalves, L. S. (1982). Weight loss and other damage to developing worker honeybees from infestation with *Varroa jacobsoni*. *J. Apic. Res.* 21, 165–167. doi: 10.1080/00218839.1982.11100535

Dietemann, V., Nazzi, F., Martin, S. J., Anderson, D. L., Locke, B., Delaplane, K. S., et al. (2013). Standard methods for *Varroa* research. *J. Apic. Res.* 52, 1–54. doi: 10.1007/978-3-319-23534-9_1

Diniz, N. M., Soares, A. E. E., Sheppard, W. S., and Del Lama, M. A. (2003). Genetic structure of honeybee populations from southern Brazil and Uruguay. *Genet. Mol. Biol.* 26, 47–52. doi: 10.1590/S1415-47572003000100008

Duranville, C., Miniggio, C., Arnold, G., and Borneck, R. (1991). Dérive des butineuses et réinfestation des colonies d'abeilles par *Varroa jacobsoni*. *La Santé L'abeille* 125, 228–235.

Ferrari, C. A., Currao, H. R., and Bessone, J. F. (2011). *La Apicultura Argentina y sus Regiones. Una Visioìn Panoraìmica*, 1st Edn, eds R. Otero and J. A. Collía (Buenos Aires: Consejo Federal de Inversiones).

Forfert, N., Natsopolou, M. E., Frey, E., Rosenkranz, P., Paxton, R. J., and Moritz, R. F. A. (2015). Parasites and pathogens of the honeybee (*Apis mellifera*) and their influence on inter-colonial transmission. *PLoS One* 10:e0140337. doi: 10.1371/journal.pone.0140337

Francis, R. M., Nielsen, S. L., and Kryger, P. (2013). *Varroa*-virus interaction in collapsing honey bee colonies. *PLoS One* 8:e57540. doi: 10.1371/journal.pone.0057540

Free, J. B. (1958). The drifting of honey-bees. *J. Agric. Sci.* 51, 294–306. doi: 10.1017/S0021859600035103

Gąbka, J. (2018). Drifting of honey bee queens returning from flights. *J. Apic. Res.* 57, 580–585. doi: 10.1080/00218839.2018.1492502

Galindo-Cardona, A., Monmany, A. C., Moreno-Jackson, R., Huertas-Dones, C., Caicedo-Quiroga, L., and Giray, T. (2012). Landscape analysis of drone congregation areas of the honey bee, *Apis mellifera*. *J. Insect Sci.* 12:122.

Galindo-Cardona, A., Quiroga, O. B., Bianchi, E., and Ayup, M. M. (2017). Primer reporte de un área de congregación de zánganos de *Apis mellifera* (Hymenoptera: Apidae) de Argentina. *Rev. Soc. Entomol. Argent.* 76, 50–53. doi: 10.25085/rsea.761207

Gasparri, N. I., and Grau, H. R. (2009). Deforestation and fragmentation of Chaco dry forest in NW Argentina (1972-2007). *For. Ecol. Manage.* 258, 913–921. doi: 10.1016/j.foreco.2009.02.024

Geographic Information System [QGIS] (2018). *QGIS Dev. Team*. Available online at: https://www.qgis.org/en/site/ (accessed September 20, 2008).

Giacobino, A., Molineri, A. I., Pacini, A., Fondevila, N., Pietronave, H., Rodríguez, G., et al. (2016). *Varroa destructor* and viruses association in honey bee colonies under different climatic conditions. *Environ. Microbiol. Rep.* 8, 407–412. doi: 10.1111/1758-2229.12410

Giacobino, A., Pacini, A., Molineri, A., Bulacio Cagnolo, N., Merke, J., Orellano, E., et al. (2017). Environment or beekeeping management: what explains better the prevalence of honey bee colonies with high levels of *Varroa destructor*? *Res. Vet. Sci.* 112, 1–6. doi: 10.1016/j.rvsc.2017.01.001

Gorelick, N., Hancher, M., Dixon, M., Ilyushchenko, S., Thau, D., and Moore, R. (2017). Google earth engine: planetary-scale geospatial analysis for everyone. *Remote Sens. Environ.* 202, 18–27. doi: 10.1016/j.rse.2017.06.031

Guzmán-Novoa, E., Eccles, L., Calvete, Y., McGowan, J., Kelly, P. G., and Correa-Benítez, A. (2010). *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. *Apidologie* 41, 443–450. doi: 10.1051/apido/2009076

Guzman-Novoa, E., and Page, R. E. Jr. (1999). Selective breeding of honey bees (Hymenoptera: Apidae) in Africanized areas. *J. Econ. Entomol.* 92, 521–525. doi: 10.1093/jee/92.3.521

Guzmán-Novoa, E., Vandame, R., and Arechavaleta, M. E. (1999). Susceptibility of European and Africanized honey bees (*Apis mellifera* L.) to *Varroa jacobsoni* Oud. in Mexico. *Apidologie* 30, 173–182. doi: 10.1051/apido:19990207

Hall, H. G., and Smith, D. R. (1991). Distinguishing African and European honeybee matrilines using amplified mitochondrial DNA. *Proc. Natl. Acad. Sci. U.S.A.* 88, 4548–4552. doi: 10.1073/pnas.88.10.4548

Harris, J. W., Harbo, J. R., Villa, J. D., and Danka, R. G. (2003). Variable population growth of *Varroa destructor* (Mesostigmata: Varroidae) in colonies of honey bees (Hymenoptera: Apidae) during a 10-year period. *Environ. Entomol.* 32, 1305–1312. doi: 10.1603/0046-225x-32.6.1305

Hou, C. S., Li, B. B., Deng, S., and Diao, Q. Y. (2016). Effects of *Varroa destructor* on temperature and humidity conditions and expression of energy metabolism genes in infested honeybee colonies. *Genet. Mol. Res.* 15:gmr8997. doi: 10.4238/gmr.15038997

Invernizzi, C., Zefferino, I., Santos, E., Sánchez, L., and Mendoza, Y. (2015). Evaluación a varios niveles del comportamiento de limpieza contra *Varroa destructor* en abejas de la miel africanizadas e italianas. *J. Apic. Res.* 54, 321–327. doi: 10.1080/00218839.2016.1159055

Jaffé, R., Dietemann, V., Crewe, R. M., and Moritz, R. F. A. (2009). Temporal variation in the genetic structure of a drone congregation area: an insight into the population dynamics of wild African honeybees (*Apis mellifera scutellata*). *Mol. Ecol.* 18, 1511–1522. doi: 10.1111/j.1365-294X.2009.04143.x

Jarvis, A., Reuter, H. I., Nelson, A., and Guevara, E. (2007). *Hole-filled seamless SRTM data (V3 ed.). International Centre for Tropical Agriculture (CIAT)*, 9. Available online: http://srtm.csi.cgiar.org

Koeniger, G., Koeniger, N., Tingek, S., and Phiancharoen, M. (2005). Variance in spermatozoa number among *Apis dorsata* drones and among *Apis mellifera* drones. *Apidologie* 36, 279–284. doi: 10.1051/apido:2005009

Koeniger, N., and Koeniger, G. (2000). Reproductive isolation among species of the genus *Apis*. *Apidologie* 31, 313–339. doi: 10.1051/apido:2000125

Koeniger, N., Koeniger, G., and Pechhacker, H. (2005). The nearer the better? Drones (*Apis mellifera*) prefer nearer drone congregation areas. *Insectes Soc.* 52, 31–35. doi: 10.1007/s00040-004-0763-z

Kralj, J., and Fuchs, S. (2006). Parasitic *Varroa destructor* mites influence flight duration and homing ability of infested *Apis mellifera* foragers. *Apidologie* 37, 577–587.

Kralj, J., and Fuchs, S. (2010). *Nosema* sp. influences flight behavior of infected honey bee (*Apis mellifera*) foragers. *Apidologie* 41, 21–28. doi: 10.1051/apido/2009046

Kraus, F. B., Koeniger, N., Tingek, S., and Moritz, R. F. A. (2005). Temporal genetic structure of a drone congregation area of the giant Asian honeybee (*Apis dorsata*). *Naturwissenschaften* 92, 578–581. doi: 10.1007/s00114-005-0044-3

Kuchling, S., Kopacka, I., Kalcher-Sommersguter, E., Schwarz, M., Crailsheim, K., and Brodschneider, R. (2018). Investigating the role of landscape composition on honey bee colony winter mortality: a long-term analysis. *Sci. Rep.* 8:12263. doi: 10.1038/s41598-018-30891-y

Le Conte, Y., Ellis, M., and Ritter, W. (2010). *Varroa* mites and honey bee health: can *Varroa* explain part of the colony losses? *Apidologie* 41, 353–363.

Lobo Segura, J. A. (2000). Highly polymorphic DNA markers in an Africanized honey bee population in Costa Rica. *Genet. Mol. Biol.* 23, 317–322. doi: 10.1590/s1415-47572000000200013

Loper, G. M., Wolf, W. W., and Taylor, O. R. (1992). Honey bee drone flyaways and congregation areas: radar observations. *J. Kans. Entomol. Soc.* 65, 223–230.

Maggi, M., Antúnez, K., Invernizzi, C., Aldea, P., Vargas, M., Negri, P., et al. (2016). Honeybee health in South America. *Apidologie* 47, 835–854.

Martin, S. J., and Medina, L. M. (2004). Africanized honeybees have unique tolerance to *Varroa* mites. *Trends Parasitol.* 20, 112–114. doi: 10.1016/j.pt.2003.12.005

McCune, B., and Mefford, M. J. (1999). *PC-ORD. Multivariate Analysis of Ecological Data. Version 5.0*. Gleneden Beach, OR: MjM Software.

Meixner, M. D., Kryger, P., and Costa, C. (2015). Effects of genotype, environment, and their interactions on honey bee health in Europe. *Curr. Opin. Insect Sci.* 10, 177–184. doi: 10.1016/j.cois.2015.05.010

Merke, J. (2016). *Dinámica Poblacional de Varroa destructor y Apis mellifera L. Como Herramienta Para la Selección de Abejas Tolerantes*. Ph.D. dissertation, National University of Mar del Plata, Mar del Plata.

Molineri, A., Giacobino, A., Pacini, A., Bulacio Cagnolo, N., Merke, J., Orellano, E., et al. (2018). Environment and *Varroa destructor* management as determinant of colony losses in apiaries under temperate and subtropical climate. *J. Apic. Res.* 57, 551–564. doi: 10.1080/00218839.2018.1475697

Mondragón, L., Spivak, M., and Vandame, R. (2005). A multifactorial study of the resistance of honeybees *Apis mellifera* to the mite *Varroa destructor* over one year in Mexico. *Apidologie* 36, 345–358. doi: 10.1051/apido:2005022

Morello, J., Matteucci, S. D., Rodriguez, A., and Silva, M. (2018). *Ecorregiones y Complejos Ecosistémicos Argentinos*, 2nd Edn. Buenos Aires: Orientacioìn Graìfica Editora S.R.L.

Mortensen, A. N., and Ellis, J. D. (2016). Managed European-derived honey bee, *Apis mellifera* sspp, colonies reduce African-matriline honey bee, A. m. scutellata, drones at regional mating congregations. *PLoS One* 11:e0161331. doi: 10.1371/journal.pone.0161331

Mortensen, A. N., Jack, C. J., and Ellis, J. D. (2018). The discovery of *Varroa destructor* on drone honey bees, *Apis mellifera*, at drone congregation areas. *Parasitol. Res.* 117, 3337–3339. doi: 10.1007/s00436-018-6035-z

Mortensen, A. N., Schmehl, D. R., Allsopp, M., Bustamante, T., Kimmel, C. B., Dykes, M. E., et al. (2016). Differences in *Varroa destructor* infestation rates of two indigenous subspecies of *Apis mellifera* in the Republic of South Africa. *Exp. Appl. Acarol.* 68, 509–515. doi: 10.1007/s10493-015-9999-8

Muerrle, T. M., Hepburn, H. R., and Radloff, S. E. (2007). Experimental determination of drone congregation areas for *Apis mellifera capensis* esch. *J. Apic. Res.* 46, 154–159. doi: 10.1080/00218839.2007.11101387

Neumann, P., and Carreck, N. L. (2010). Honey bee colony losses. *J. Apic. Res.* 49, 1–6. doi: 10.3896/IBRA.1.49.1.01

Neumann, P., Moritz, R. F. A., and Mautz, D. (2000). Colony evaluation is not affected by drifting of drone and worker honeybees (*Apis mellifera* L.) at a performance testing apiary. *Apidologie* 31, 67–79. doi: 10.1051/apido:2000107

Neumann, P., Radloff, S. E., Pirk, C. W. W., and Hepburn, R. (2003). The behaviour of drifted Cape honeybee workers (*Apis mellifera capensis*): predisposition for social parasitism? *Apidologie* 34, 585–590. doi: 10.1051/apido:2003048

Nolan, M. P., and Delaplane, K. S. (2017). Distance between honey bee *Apis mellifera* colonies regulates populations of *Varroa destructor* at a landscape scale. *Apidologie* 48, 8–16. doi: 10.1007/s13592-016-0443-9

Olson, D. M., Dinerstein, E., Wikramanayake, E. D., Burgess, N. D., Powell, G. V. N., Underwood, E. C., et al. (2001). Terrestrial ecoregions of the world: a new map of life on earth: a new global map of terrestrial ecoregions provides an innovative tool for conserving biodiversity. *BioScience* 51, 933–938. doi: 10.1641/0006-3568(2001)051[0933:TEOTWA]2.0.CO;2

Oyarzabal, M., Clavijo, J., Oakley, L., Biganzoli, F., Tognetti, P., Barberis, I., et al. (2018). Unidades de vegetación de la Argentina. *Ecol. Austral* 28, 1–156. doi: 10.25260/EA.18.28.1.0.399

Paar, J., Oldroyd, B. P., Huettinger, E., and Kastberger, G. (2002). Drifting of workers in nest aggregations of the giant honeybee *Apis dorsata*. *Apidologie* 33, 553–561. doi: 10.1051/apido

Pfeiffer, K. J., and Crailsheim, K. (1998). Drifting of honeybees. *Insectes Soc.* 45, 151–167. doi: 10.1007/s000400050076

Punchihewa, R. W. K., Koeniger, N., and Koeniger, G. (1990). Congregation of *Apis cerana indica* Fabricius 1798 drones in the canopy of trees in Sri Lanka. *Apidologie* 21, 201–208. doi: 10.1051/apido:19900305

Quezada-Euan, J. J. G., and Jesus May-Itza, W. D. (2006). Partial seasonal isolation of African and European-derived *Apis mellifera* (Hymenoptera: Apidae) drones at congregation areas from subtropical Mexico. *Ann. Entomol. Soc. Am.* 94, 540–544. doi: 10.1603/0013-8746(2001)094[0540:psioaa]2.0.co;2

Quinn, G. P., and Keough, M. J. (2002). *Experimental Design and Data Analysis for Biologists*, 1st Edn. New York, NY: Cambridge University Press.

R Core Team (2017). *R: A Language and Environment for Statistical Computing.* Vienna: R Foundation for Statistical Computing.

Rivera-Marchand, B., Oskay, D., and Giray, T. (2012). Gentle Africanized bees on an oceanic island. *Evol. Appl.* 5, 746–756. doi: 10.1111/j.1752-4571.2012.00252.x

Rosenkranz, P. (1999). Honey bee (*Apis mellifera* L.) tolerance to *Varroa jacobsoni* Oud. in South America. *Apidologie* 30, 159–172. doi: 10.1051/apido:19990206

Ruttner, F. (1966). The life and flight activity of drones. *Bee World* 47, 93–100. doi: 10.1080/0005772X.1966.11097111

Sheppard, W. S., Rinderer, T. E., Mazzoli, J. A., Stelzer, J. A., and Shimanuki, H. (1991). Gene flow between African-and European-derived honey bee populations in Argentina. *Nature* 349, 782–784. doi: 10.1038/349782a0

Southwick, E. E., and Buchmann, S. (1995). Effects of horizon landmarks on homing success in honey bees. *Am. Nat.* 146, 748–764. doi: 10.1086/285823

Strang, G. E. (1970). A study of honey bee drone attraction in the mating response. *J. Econ. Entomol.* 63, 641–645. doi: 10.1093/jee/63.2.641

Wattanachaiyingcharoen, W., Wongsiri, S., Oldroyd, B. P., Wattanachaiyingcharoen, W., Wongsiri, S., and Oldroyd, B. P. (2008). Aggregations of unrelated *Apis florea* colonies. *Apidologie* 39, 531–536.

Whitfield, C. W., Behura, S. K., Berlocher, S. H., Clark, A. G., Johnston, J. S., Sheppard, W. S., et al. (2006). Thrice out of Africa: ancient and recent expansions of the honey bee, *Apis mellifera*. *Science* 314, 642–645. doi: 10.1126/science.1132772

Yañez, O., Jaffé, R., Jarosch, A., Fries, I., Robin, F. A. M., Robert, J. P., et al. (2012). Deformed wing virus and drone mating flights in the honey bee (*Apis mellifera*): implications for sexual transmission of a major honey bee virus. *Apidologie* 43, 17–30. doi: 10.1007/s13592-011-0088-7

Youngsteadt, E., Appler, R. H., López-Uribe, M. M., Tarpy, D. R., and Frank, S. D. (2015). Urbanization increases pathogen pressure on feral and managed honey bees. *PLoS One* 10:e0142031. doi: 10.1371/journal.pone.0142031

Zmarlicki, C., and Morse, R. A. (1963). Drone congregation areas. *J. Apic. Res.* 2, 64–66. doi: 10.1080/00218839.1963.11100059

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The Process and Outcome of the Africanization of Honey Bees in Mexico: Lessons and Future Directions

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The Africanization of honey bees (*Apis mellifera* L.) in the Americas is among the most extensive insect invasions in the world, with large-scale effects on the economy and ecology of a whole continent. Africanized honey bees (AHBs) are a distinctive lineage of *A. mellifera*, resulting of the extensive admixture between African subspecies (*A. m. scutellata* and *A.m. adansonii*) with resident European stocks of honey bees introduced into the Americas. Despite its great importance, to date, the outcome of Africanization of honey bees has not been evaluated in detail. In this article we use the case of Mexico, one of the top beekeeping countries in the world, to assess the effects of Africanization of honey bees and its outcome. There is evidence of African genes in honey bee populations across Mexico, with prevalence in the tropical areas and less so in temperate ones. The Africanization of honey bees resulted in lower honey yields per hive in temperate climates of Mexico, but this has not been assessed in the tropical regions. Mexico's total honey production and exports at the start of the Africanization process decreased, but today, they have partially rebounded and have remained stable. As in all countries where Africanization has occurred, the defensive behavior of honey bees in Mexico increased but notably, stinging incidents involving humans have been relatively insignificant (0.23 fatalities per million people). Ecologically, AHBs seem to have posed limited impact on the native apifauna and have contributed to pollination of major economic crops, but more studies are needed to evaluate the overall effect. AHBs can be potentially more resistant to parasites and diseases and worth of note is that AHBs in Mexico resulted in a new generation of beekeepers that propelled management and selective breeding. In general, the evidence suggests that the Africanization of honey bees in Mexico has had a less severe impact than originally predicted. We suggest some lines for future directions that may help to better understand the effects, make sustainable use and ameliorate the negative characteristics of AHBs.

Keywords: ***Apis mellifera*, Africanized honey bees, native bee, adaptation, invasive organism, impact, Mexico**

INTRODUCTION

The Western honey bee (*Apis mellifera* L.) originated in the Old World (Europe, Africa and the near East), where influenced by different selective environments, diversified into several subspecies (Ruttner, 1988). In the Americas, early European settlers introduced Western European *A. m. mellifera* and *A. m. iberiensis,* followed by later introductions of Eastern European races, mainly *A. m. ligustica* (Whitfield et al., 2006). Thus, up to 1956, honey bees of predominantly European-descent existed in the Americas. In that year, Brazilian researchers introduced queens of *A. m. scutellata* and *A.m. adansonii*, from South Africa and Tanzania to the state of São Paulo, Brazil, to develop a selective breeding program (Kerr, 1967). European honey bees (EHBs) kept in Brazil were not well adapted to tropical conditions, and a hybrid bee could be better suited for these regions (Kerr, 1967). An accident caused the release of pure African colonies, which interbred with locally existing EHBs, thereof, originating the so-called Africanized honey bee (AHB) through the process of Africanization (Nogueira-Neto, 1964; Rinderer and Hellmich, 1991).

The process of Africanization is one of the most dramatic invasion events by any animal species (Page, 1989; Clarke et al., 2002). In spite of many attempts to stop the advance of AHBs, they expanded rapidly and produced large feral populations, which in the course of 30 years, colonized most of the Americas displacing resident EHBs, except in temperate areas, presumably because of reduced adaptation to these environments (Rinderer and Hellmich, 1991; Schneider et al., 2004). In their northward and southward advance, AHBs disrupted beekeeping in many countries of South and Central America, in part, because the beekeeping industry was not extensively developed and only low concentrations of EHB colonies existed (Rinderer and Hellmich, 1991; Rinderer et al., 1991). In contrast, Mexico has one of the highest concentrations of managed EHB colonies worldwide, and a great diversity of climates, ecosystems and beekeeping regions (Labougle and Zozaya, 1986; Quezada-Euán, 2007). In Mexico, beekeeping is a major activity of great economic and social importance, making the country the sixth world's largest honey producer and the third largest honey exporter (Programa Nacional para el Control de la Abeja Africana [PNCAA], 2010). The organization of Mexican beekeepers and the management techniques were more developed than in much of Central and South America (Labougle and Zozaya, 1986; Guzman-Novoa, 1996; Programa Nacional para el Control de la Abeja Africana [PNCAA], 2010). Initial predictions of the effects of Africanization were highly pessimistic, foreseeing a collapse of beekeeping. More than 60 years after the start of Africanization of honey bees, no account had been conducted on the effect and consequences of AHBs in the Americas. Mexico is a good country to assess the impact of colonization, adaptation and husbandry of this invading insect. Some of the most comprehensive studies about the defensive behavior, genetics of the Africanization process, ecology and selective breeding of honey bee populations, have been conducted in Mexico. In this review article, we document what is known about different aspects of the biology of AHB populations established in Mexico, the spread across different ecological environments, and how their behaviors and other traits have contributed to their adaptation and impact on human health, the beekeeping industry and ecosystems.

HISTORY AND GENETIC MAKEUP OF AHBs IN MEXICO

The genetic nature of AHBs had been strongly debated (Hall and Muralidharan, 1989; Smith et al., 1989; Rinderer and Hellmich, 1991). The expansion front and feral populations of AHBs in South and Central America showed little contribution of EHBs (Smith et al., 1989). This evidence suggested that AHBs were mostly of African ancestry and that there may have been reproductive and genetic mechanisms preventing hybridization with EHBs (Harrison and Hall, 1993). However, in opposition to that view, it was argued that asymmetric hybridization and, thus, little evidence of European genes in feral populations would be expected if European colonies were vastly outnumbered by AHBs (Rinderer and Hellmich, 1991). Studies in South America revealed that the asymmetry of the process of Africanization was reinforced by several reproductive strategies of AHBs such as a larger investment in swarms per year, a significantly higher production of drones, drone and queen parasitism of EHB colonies, higher absconding rates, assortative mating and, rapid ontogenetic development of AHBs queens (Rinderer et al., 1985a,b, 1987; Hellmich, 1991; Rinderer and Hellmich, 1991; Danka et al., 1992; Schneider and McNally, 1992; Vergara et al., 1993; DeGrandi-Hoffman et al., 1998b; Taylor, 1999; Quezada-Euán and May-Itza, 2001; Schneider et al., 2004). The course of Africanization in Mexico was crucial to test those two opposing hypotheses because in Mexico, the advancing Africanized front would encounter a large population of managed EHB colonies, thus, both being for the first time in comparable numerical conditions (Hellmich and Rinderer, 1991; Taylor, 1999; Clarke et al., 2002).

AHB swarms were first detected in 1986 in the state of Chiapas, Mexico, near the border with Guatemala (Fierro et al., 1987; Moffett et al., 1987). Swarm trap lines were established between 1986 and 1987 in all states of Mexico to evaluate the movement of AHBs across the country, using morphometric and allozyme analyses (Daly and Balling, 1978; Fierro et al., 1987). Chiapas is a state clearly marked by two regions, the tropical lowlands and the temperate highlands and differences in the rate of movement of colonizing swarms were found in both climate types. Only 3% of the colonizing swarms moved at altitudes above 400–500 m, but there was a fast migration and occupation of the tropical lowlands, where swarms moved at an average of 400 km/year (Quezada-Euán, 2007). It was also evident that the large mountain ranges in the South of the country and those surrounding the high plateau posed a barrier for the movement of swarms into this region in which they were only found until 1990 (Quezada-Euán, 2007). Therefore, the Africanization of honey bees in Mexico rapidly occurred along two fronts of migrating swarms that moved from Central America across the tropical Pacific and Gulf

Coast lowlands and into the Yucatan Peninsula. Notably, genetic differences were found in swarms composing both colonizing fronts. Morphometric and allozyme analysis of swarms of the Gulf coast showed that they had higher frequency of African markers compared to the ones of the Pacific coast (Labougle et al., 1989). Long-term studies of the immigrant AHBs show that swarm numbers may wane some years after initial colonization (Roubik, 1989).

The Africanization of apiaries in Mexico was first studied in the Yucatan Peninsula (Rinderer et al., 1991; Quezada-Euán et al., 1996). Managed colonies showed rapid evidence of African gene introgression, but also extensive hybridization with resident honey bees of European descent (Rinderer et al., 1991; Quezada-Euán and Paxton, 1999). Moreover, although the majority of feral colonies in Yucatan had African mitotypes (mtDNA) and wing lengths not different from those of *A. m. scutellata*, notably, 20% had European mtDNA and European morphometrics, which indicated a contribution of the managed EHB population to the feral AHBs (Quezada-Euán and Hinsull, 1995). The presence of European markers in the feral bee population of Yucatan was explained by the large population size of resident EHBs that existed in that region at the arrival of AHBs (Quezada-Euán and Hinsull, 1995).

Similar results have been found in the temperate regions of Northern of Mexico. There, the frequency of African and European morphotypes and mitotypes was analyzed at three different altitudes (Medina-Flores et al., 2014, 2015). It was found that the frequency of colonies with African or European mitotypes and morphotypes varied significantly between regions, with results indicating a higher degree of Africanization in warm semi-dry and subtropical regions. Conversely, the highest frequency of colonies with European morphotype and mitotype occurred in temperate regions, supporting the notion that the environment and climate influence the outcome of Africanization of honey bee colonies (Rinderer et al., 1991; Sheppard et al., 1991a). Likewise, at higher altitudes, more colonies have European genotype compared with colonies established in tropical and low-altitude regions (Quezada-Euán et al., 2003). Conversely, in tropical Veracruz, Kraus et al. (2007) did not find evidence of lower frequency of African markers in honey bee colonies at high altitudes in Veracruz, Mexico. Nonetheless, it is possible that the bees collected from feral colonies at high altitudes in their study had been derived from colonies transported by migratory beekeepers from low lands to high lands, a common practice carried out with thousands of hives every year to take advantage of different blossoms in the regions where the study by Kraus et al. (2007) was conducted.

Climate and genetic differences of the Africanization fronts (Quezada-Euán, 2007) may explain differential Africanization rates in the North of Mexico. In Northwestern Mexico, frequencies of up to 56% of European mitotypes were still found in apiaries in 2004 (Zamora et al., 2008). In contrast, in Northeastern Mexico, only 30% of the colonies sampled had European mtDNA (Silva-Contreras et al., 2019). These results support the notion that the movement of AHBs across the Eastern region was more intense and preserved better the African lineage compared with the front in the Western region (Quezada-Euán, 2007).

Recently, a comprehensive study including 500 colonies collected from the five beekeeping regions of Mexico yielded a better picture of the current genotypic composition of honey bees in the country (Domínguez-Ayala et al., 2016). About half of the colonies sampled (51.5%) had African mitotypes. The highest frequency of African mitotypes was found in the tropical beekeeping region of the Gulf coast (69.8%), followed by the Yucatan Peninsula (63.8%), and the Pacific coast region (63.1%). The lowest frequency of African mitotypes was observed in the Northern region (24.9%), where European mitotypes predominated. European mitotypes were also more frequently found in the high plateau region. Morphometric data showed a similar pattern, with larger frequencies of European morphometrics in colonies of the high plateau and North and an opposite trend in the tropics (**Figure 1**). Interestingly, only 8% of the samples had both European mitotype and morphology, suggesting that the majority of honey bee populations in Mexico show a degree of African gene introgression. This study confirmed that as originally proposed, the degree of Africanization is climate-driven given the differential adaptation of EHBs and AHBs to temperate and tropical environments, respectively (Sheppard et al., 1991a). It is likely that traits making AHBs successful tropical honey bees (high investment in swarms and brood, eclectic use of lodgings, reduced ability to control nest temperature, and other traits) could severely limit their progress in temperate areas (Sheppard et al., 1991a; Schneider et al., 2004).

Presently, the process of Africanization of Mexico's honey bees seems to have stabilized to a large extent (Domínguez-Ayala et al., 2016). In general, the honey bees of Mexico show evidence that hybridization has occurred extensively and that climate drives introgression of African genes into local populations. As a consequence, there are two clear populations, one mainly composed by AHBs on the tropical beekeeping regions of the Yucatan Peninsula, the Gulf of Mexico and the Pacific Coast and, another where EHBs predominate, in the temperate beekeeping regions of the high plateau and the North of the country. In general, the results of Mexico are in agreement with what has also been found in South America and the United States. Hybrid honey bee populations occur near the historic origin of AHBs in Brazil (Sheppard et al., 1991b). In Southern Texas, 23 years after the first report of AHBs, nearly 90% of the colonies had *A. m. scutellata* maternal ancestry, but nuclear DNA markers revealed little change in African genes compared to populations from the period 1991–2001 (Rangel et al., 2016). Thus, the general outcome of Africanization in Mexico and elsewhere indicates the existence of a hybrid swarm (Rinderer and Hellmich, 1991) with different contribution of African and European genes depending on climatic conditions.

THE ECOLOGICAL IMPACT OF AHBs IN MEXICO

During the colonization of the Americas, AHBs expanded across some 16 million Km^2 (Roubik, 1987). Most of this area was

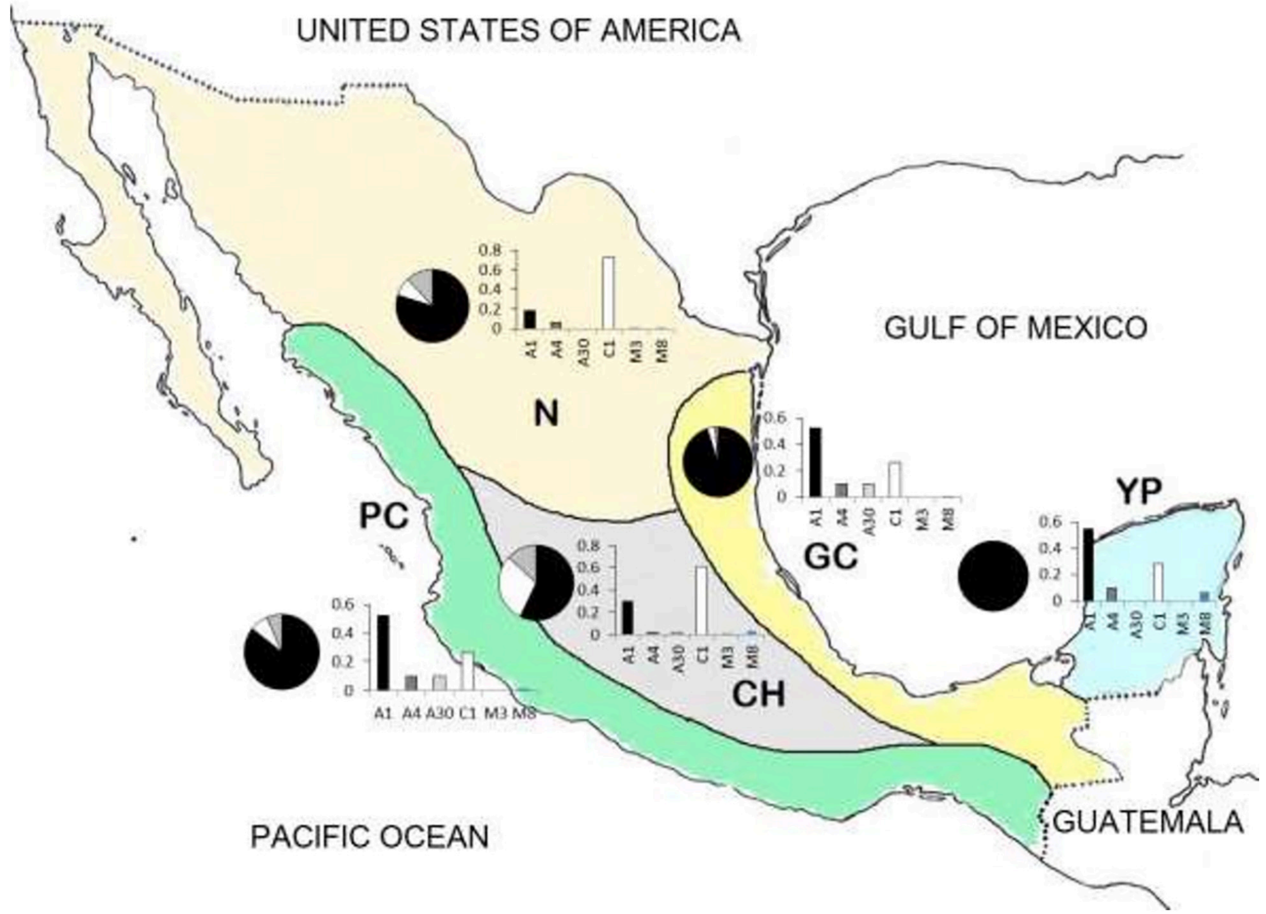

FIGURE 1 | Map of Mexico showing the five main beekeeping regions of the country: North (N), Central highlands (CH), Pacific coast (PC), Gulf coast (GC), and Yucatan Peninsula (YP). The relative frequencies of mitotypes (bar graphs) and morphotypes (pie graphs) in each region show African in black, European in white and intermediate in gray. Letters refer to three different mitotypes of *A. mellifera*, namely, African (A), West-European (M), and East-European (C) (data from Domínguez-Ayala et al., 2016).

devoid of honey bees, as EHBs never established feral populations (Quezada-Euán and Hinsull, 1995; Roubik, 2000). However, by the end of last century, the tropics and subtropics contained a large population estimated in approximately one trillion colonies of honey bees of African descent from Northern Argentina to Mexico. They may consume two billion Kg of pollen and 20 billion Kg of nectar annually (Roubik, 1989). As dramatic as this may seem, compared with the rapid and hard impact on beekeeping, the influence and changes caused by the huge population of AHB colonies in the native ecosystems have been slow and difficult to detect (Roubik, 1989). There are several ways in which exotic bees can affect native bees (Goulson, 2003). These include: competition with native flower visitors for floral resources, competition with native organisms for nest sites, changes in seed set of native plants (either increases or decreases) and exotic weeds, plus transmission of parasites or pathogens to native organisms (Goulson, 2003).

The honey bee is well adapted to exploit a wide range of floral resources. Colonies are generalists and can rapidly recruit to and exploit more efficiently these resources than native bees (Roubik, 1989). Thus, a first impact could be on competition for floral resources and the displacement of native bees. Notably, although AHBs can be present in large numbers on flower patches, they do not display aggression toward other bees. Indeed, aggression is more frequent the other way around and among AHBs (Roubik and Villanueva-Gutiérrez, 2017). Rather, AHBs may displace native species by numerical advantage (Roubik, 1989). Surprisingly, long-term studies from Central America and Southern Mexico have revealed that the Neotropical bee-plant assemblages in those regions do not exist in a delicate balance and that the introduction of honey bees do not invariably upset the system (Roubik, 2000). Evidence for high resiliency of native bee populations (solitary or social) to the arriving AHBs was obtained from a long-term study in Quintana Roo, Mexico. There, native bees abandoned some resources and changed their pollen preferences to other resources after the arrival of AHBs (Roubik, 2009; Roubik and Villanueva-Gutiérrez, 2009). The native bees also shifted their foraging times to avoid competition with AHBs. Surprisingly, solitary bee abundance also increased in some years after feral AHBs were well established, so indirect benefits from the invader, such as greater pollination and abundance of certain native plants used as food by native bees could be occurring (Roubik and Villanueva-Gutiérrez, 2009). However, native bee flexibility on the use of floral resources may be constrained by the abundance and diversity of the local plant assemblage (Roubik, 2009). This means that food competition among exotic and native bees could be more intense when floral resources are limited and the resiliency of native communities could then be more compromised (Roubik, 1989, 2009). Additionally, recent studies have shown that the effect of AHBs on specialized bees and plants that depend on them may be more severe. A study from the United States revealed that the oligolectic bees *Perdita meconis* and *Eucera quadricincta* have disappeared in Southern Utah as a result of AHBs monopolizing *Arctomecon humilis*, the main pollen source for females of those species (Portman et al., 2018). Oligolectic bee species being unable to switch host-plants, can be left with little food needed to rear their progeny (Portman et al., 2018).

By affecting the diversity of native floral visitors, AHBs can also have an impact on the composition of local flora, but this has been seldom analyzed. One study showed that floral visitation by AHBs affects male fitness and probably fruit and seed production of the tropical tree *Clusia arrudae,* but without affecting the resource sought by native pollinators (Do Carmo et al., 2004). In Brazil, applying networks theory, Santos et al. (2012) found that AHBs induced significant changes in the structure of native pollination networks, mainly by making them more cohesive and monopolizing many interactions. Another network study conducted in four regions of Mexico showed that areas high in endemic species can have more specialized plant-bee interactions, and thus can be more susceptible to the effect of AHBs (Ramírez-Flores et al., 2015).

Conversely, AHBs can provide good pollination services and increase the productivity of plants and cash crops that do not require specialized pollinators. Coffee production has increased in the Americas and Mexico, probably as a result of AHBs increased floral visitation (Roubik, 2002; Vergara and Badano, 2009). On economically important Mexican crops like avocado, tomato, chilli and, physic nut, AHBs can be frequent visitors even after heavy insecticide spraying, when local apifauna wanes (Macías-Macías et al., 2009; Pérez-Balam et al., 2012; Romero and Quezada-Euán, 2013; Landaverde-González et al., 2017). Furthermore, in the light of pollinator declines (Potts et al., 2010), AHBs may act complementing the pollination services of scarce native floral visitors. One study conducted in Yucatan, Mexico, on the buzz-pollinated annatto (*Bixa orellana*), showed a high individual efficiency of the native stingless bee *Melipona beecheii*, compared with AHBs (Caro et al., 2017). However, AHBs were present in significantly higher numbers and were observed gleaning pollen previously extracted by *M. beecheii*, acting as a commensal. As *M. beecheii* is normally rare in this region of Mexico, AHBs may compensate the numeric lack of the original pollinator (Caro et al., 2017). On the Pacific coast of Mexico, temporal variation in the pollinator community and in the pollination efficiency of the main pollinators of *Cucurbita moschata* was found (Delgado-Carrillo et al., 2018). In the wet season, solitary native bees of the genus *Peponapis* were the most frequent and effective pollinators of *C. moschata*, whereas in the dry season, *Peponapis* bees were scarce. However, AHBs became the most frequent floral visitor providing an effective pollination service in this season (Delgado-Carrillo et al., 2018). Evidently, more studies are needed to understand the dynamics of AHBs with native pollinators and plants that require specialized pollination.

Nest site competition between AHBs and native stingless bees seems minimal because the former accept cavities with large openings that are not suitable for most native species and they also frequently build nests in the open (Roubik, 1989). However, the effect on other cavity nesting animals (birds and mammals) can be more severe (Efsthation and Kern, 2016). Arguably, total competition for nest sites and food could become more intense between AHBs than between the honey bees and native bees. Such competition among AHBs may be one factor contributing to curb their rate of population growth and the production of swarms (Roubik, 1989).

One less evident positive impact of honey bee Africanization in Mexico is the collateral impulse of stingless beekeeping. Before the arrival of AHBs, stingless beekeeping, an ancient activity dating from pre-Columbian times, was at the verge of extinction (Quezada-Euán et al., 2001). However, after 1986, to avoid stinging incidents, apiaries had to be relocated in the forests, further away from human settlements where they were normally kept. This opened a niche for the buildup of stingless bee backyards or meliponarios, which posed no risk to people (Quezada-Euán, 2018). New techniques started to be developed and applied, which propelled the modernization and rebirth of stingless beekeeping in Mexico (Quezada-Euán, 2018).

Native bee predators may have benefited by the sudden abundance of feral AHBs colonies. Army ants, for instance, are some of the major predators of stingless bees, but these have evolved different mechanisms to efficiently defend their colonies (Quezada-Euán, 2018). Instead, army ants frequently decimate AHBs colonies in apiaries, whilst only a few stingless bee colonies are lost in meliponarios (Quezada-Euán, 2018).

The Africanization of honey bees has resulted in extensive and more frequent interactions between honey bees and native bees (Roubik, 1989). One potential problem of such close interactions is the transmission of parasites and diseases, the dynamics of which are still not well understood. It is known that AHBs can be more resistant to some parasites like the mite *Varroa destructor* (Guzman-Novoa et al., 1999; Martin and Medina-Medina, 2004) that has decimated colonies of EHBs elsewhere (Guzman-Novoa et al., 2010). AHBs can also be more resistant than EHBs to viral infections (Hamiduzzaman et al., 2015). Seemingly, most honey bee parasites are not transmissible to native stingless bees and no major epidemics have been recorded in the latter (Quezada-Euán, 2018). However, several viruses found in honey bees have recently been detected in other bee species (McMahon et al., 2015; Tehel et al., 2016), raising the possibility of spill-over from AHBs to native bees. Honey bee viruses have been found in native stingless bees from Brazil (Ueira-Vieira et al., 2015) and Mexico (Guzman-Novoa et al., 2015). Surprisingly, although honey bee viruses have been found to replicate in Mexican stingless bees, no signs of disease have been found in colonies (Tapia-González et al., 2019; Morfin et al., 2020). Notably, a study conducted in the Brazilian island of Fernando de Noronha, found ubiquitous presence of deformed wing virus type C (DWV-C) in *M. subnitida* colonies, but rarity in *A. mellifera*, which suggested limited viral exchange between these two species (De Souza et al., 2019). It is possible that the so-called honey bee viruses may be generalist bee viruses or insect viruses and thus they could spread also from native bees to honey bees. More research is thus needed to clarify the issue of potential pathogen spillover between different bee species and other pollinators. AHBs seem to be also tolerant to other parasites that are damaging to EHBs, such as *Nosema ceranae* (Fleites-Ayil et al., 2018), and this microsporidian has also been found to multiply in *M. colimana*, an endemic stingless bee from Jalisco, Mexico (Macías-Macías et al., 2020). However, not much more is known about the potential virulence of honey bee pathogens in native bees. Clearly, the nature and extent of disease spillover from and between AHBs and native bees is still in early stages and deserves further investigation.

THE ECONOMIC IMPACT OF AHBs IN MEXICO

Honey Production

Honey production is a complex trait affected by many factors including floral resources, climate, colony populations and beekeeping practices. However, under similar environmental conditions of food resources, climate and management, differences in honey yields between colonies are directly associated to the population size of colonies, the length of life of forager bees and the foraging efficiency of bees (Woyke, 1984; Guzman-Novoa and Gary, 1993; Becerra-Guzmán et al., 2005). Therefore, differences in these traits between bee ecotypes could partially explain differences in honey production.

For reproduction rates, it is well established that AHBs reproduce at a faster rate than EHBs (Winston, 1979, 1992). For length of life and foraging efficiency, few studies have analyzed and compared AHBs and EHBs in a Mexican environment. Becerra-Guzmán et al. (2005) established colonies of both bee ecotypes in Tonatico, Mexico, and co-fostered marked AHBs and EHBs in the same colonies to study genotypic effects on their lifespan and foraging behavior. Both, EHBs and AHBs varied for length of life within a range of 20–26 days, but there was no significant difference for this trait between the two types of bees. Hive environment greatly influenced the lifespan of both bee types, indicating that genetic effects have less influence on this trait than environmental effects. By comparison, the length of life of worker bees of colonies from South America was reported to vary and no clear difference for this trait was found between EHBs and AHBs (Winston and Katz, 1981), which is similar to what Becerra-Guzmán et al. (2005) concluded in Mexico. What differed between the two bee types in the study by Becerra-Guzmán et al. (2005) was their foraging life; AHBs had shorter foraging lives (9 ± 0.4 days) than EHBs (12 ± 0.5 days), which could have a significant impact on food stores and honey yields of colonies. Additionally, work done in the same region of Mexico, showed that AHBs and EHBs did not differ in foraging force, number of total foraging trips and amount of nectar or pollen collected per individual bee, but they differed in how the foraging force was allocated. AHBs dedicated a significantly lower proportion of nectar foragers and a significantly higher proportion of pollen foragers in comparison with EHBs (Neuman, 2001). Other studies conducted in South America have reported that when different components of foraging behavior are considered altogether, such as the number of trips to flowers, as well as the quantity and quality of the nectar (sugar concentration of the nectar) transported to the hive, there are no differences between AHBs and EHBs for the amount of calories that each individual bee dedicated to the collection of nectar contributes to its colony (Rinderer et al., 1985a; Pesante et al., 1987; Rinderer and Collins, 1991). However, it has been well established that AHBs collect more pollen than EHBs because they allocate a higher proportion of their foraging force to collect pollen than bees of EHB colonies. Danka et al. (1987) compared the proportion of foragers dedicated to pollen foraging in colonies of both bee types in Venezuela, and found that more than 30% of the bees from AHB colonies performed pollen trips, while less than 15% of the bees from EHB colonies did it. Fewell and Bertram (2002) corroborated these findings in Arizona.

The consistency of results on the length of life, foraging efficiency and foraging strategy of honey bees in studies conducted in Mexico and South America, indicate that the main differences for these traits between AHBs and EHBs are longer foraging lives and higher proportions of foragers dedicated to nectar collection in EHB colonies. These differences in foraging life and foraging strategy give an advantage to EHBs over AHBs for honey production, and an advantage to AHBs for increased reproduction and colonization of new environments.

Honey production has decreased in all countries where AHBs have become established in at least half of their territory, with the exemption of Brazil (Gonçalves et al., 1991; Rinderer and Collins, 1991; Guzman-Novoa and Page, 1994b; Caron, 2001). This is one of the reasons why there is controversy about whether these bees are better suited for honey production than EHBs. The controversy has not been totally resolved because few side by side studies between colonies of the two bee eco-types have been conducted to compare them for honey yields (Kerr, 1967; Rinderer et al., 1985b; Spivak et al., 1989). Moreover, the number of colonies used in those studies (<15 per treatment) was low for valid statistical comparisons (small sample sizes) because the variation for honey yields among colonies is high (Uribe-Rubio et al., 2003). Additionally, the results of the above studies have been inconsistent. For example, Kerr (1967) found that AHBs were more productive than EHBs, Rinderer et al. (1985b) concluded that EHBs produced more honey than AHBs, and Spivak et al. (1989) did not find differences between colonies of the two bee types. Clearly, studies with a larger number of colonies conducted in different environments are necessary to obtain consistent results and to draw firm conclusions about the effect that the Africanization of honey bee populations has on honey production. Considering the above, Guzman-Novoa and Uribe-Rubio (2004) compared the honey yields of 118 EHB, 80 AHB and 84 hybrid colonies in a temperate region of Mexico. EHB colonies yielded over 30% more honey than AHB and hybrid colonies of Africanized maternal ancestry, which was significant. Hybrid bee colonies of European maternal ancestry produced slightly more honey than EHB colonies, but their yields were not significantly different. Additionally, hybrid colonies of Africanized maternal ancestry produced similar yields of honey to those of AHB colonies (**Figure 2**). These results demonstrate that colonies of primarily African ancestry produce less honey than colonies of primarily European ancestry. Moreover, they suggest maternal or dominance effects for honey production, and demonstrate that Africanization decreases honey yields of honey bee colonies, at least under the temperate environment of Mexico where they were tested. Uribe-Rubio et al. (2003), working in the same geographical area where the study by Guzman-Novoa and Uribe-Rubio (2004) was conducted, had previously found in a large study involving 416 colonies, that colonies having bees with African mtDNA produced significantly less honey than those having bees with European mtDNA. Therefore, it does not seem advantageous to work with populations of honey bees with a high degree of Africanization if honey yields are the primary objective of beekeeping operations. Mexican beekeepers have to work with AHBs because they are present and well adapted to most beekeeping regions in Mexico, but perhaps they can reduce the level of Africanization of colonies through selective breeding.

The reasons why EHB and AHB colonies vary for stored honey that can be harvested are not well known, but they may be related with the behavioral adaptations of the two bee ecotypes to different environments in the regions of the world where they evolved (Page, 1989). AHB colonies collect more pollen and less nectar compared to EHB colonies of similar strength because they allocate less bees for nectar collection than EHB colonies (Danka et al., 1987; Pesante et al., 1987). Additionally, AHB colonies tend to keep lower food stores than EHB colonies because they use more of their food resources for reproduction than EHB colonies (Pesante et al., 1987; Winston, 1992). Another factor that may explain, at least in part, why AHB colonies produce on average less honey that EHB colonies, is a higher swarming frequency (Winston, 1992). Colonies swarming before or during the nectar flow season will be less populated than colonies that do not swarm and therefore will be less productive. Also, absconding (hive abandonment), a tactic of tropical bees to avoid starvation and predation, can partially explain the lower mean honey yields per colony in Africanized areas of Mexico (Guzman-Novoa et al., 2011).

The above studies have shown that at the colony level, AHBs seem to produce less honey per hive than EHBs, at least under temperate environments in Mexico. At the country level, it is more difficult to establish the actual impact that the Africanization of honey bees has had on honey production. Nevertheless, coincidentally with the arrival and spread of AHBs in Mexico, the production of honey in the

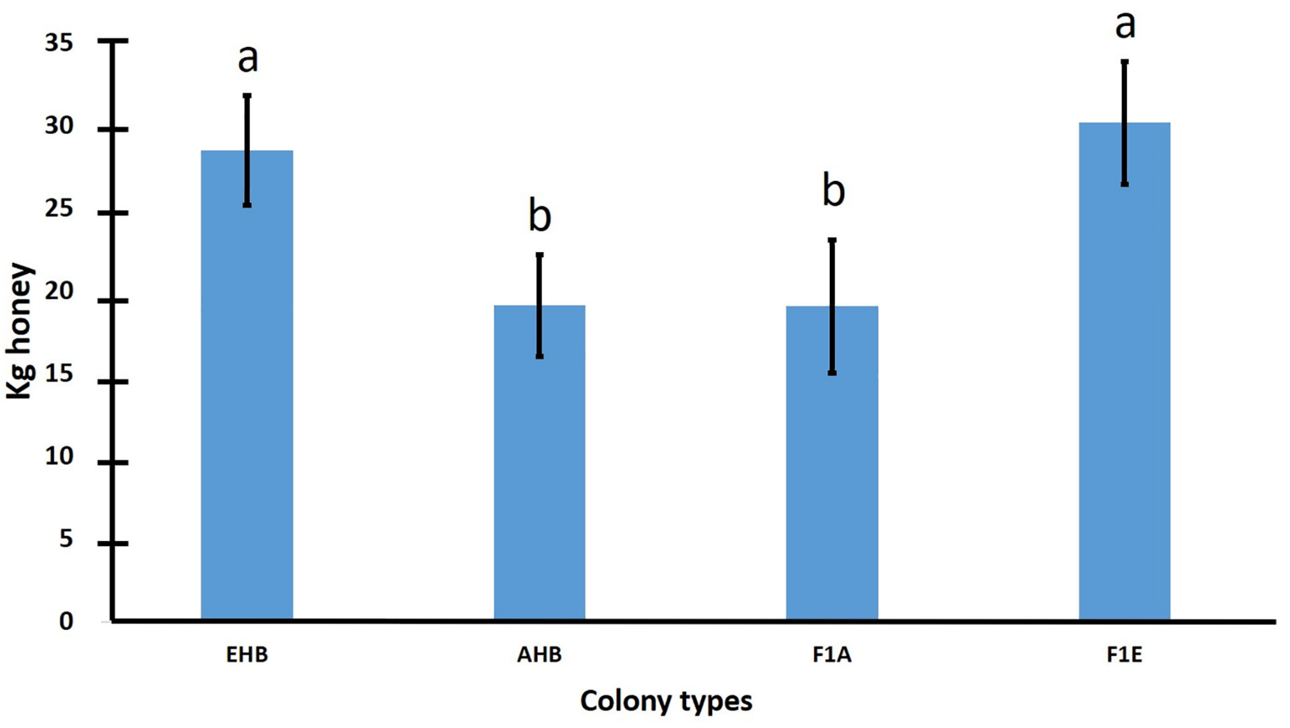

FIGURE 2 | Mean (± SE) honey yield per hive for 282 honey bee colonies of European, Africanized and hybrid genotypes (data from Guzman-Novoa and Uribe-Rubio, 2004).

country, progressively decreased within 10 years after that event (Guzman-Novoa et al., 2011).

Even when honey production decreased in Mexico during the first decade after the arrival of AHBs, the reduction was not as severe as in Venezuela or Central America (Hellmich and Rinderer, 1991; Guzman-Novoa, 1996; Caron, 2001), probably because there is better beekeeping infrastructure in Mexico than in those countries (Guzman-Novoa, 1996, 2004; Programa Nacional para el Control de la Abeja Africana [PNCAA], 2010). In 1986, the year when AHBs arrived in Mexico, the country's honey production was 74,613 tons, whereas honey exports exceeded 48,000 tons (Sader, 2020). Ten years after their arrival, in 1996, honey production was estimated to be under 49,000 tons, while honey exports were less than 27,000 tons, which is equivalent to 66 and 56% of the honey that was produced and exported, respectively, the year of arrival of AHBs to the country. Twenty years after the arrival of AHBs, in 2006, honey production exceeded 55,000 tons and honey exports reached 29,000 tons (Sader, 2020), which reflects a slight increase relative to 10 years before (**Figures 3**, **4**), despite the fact that new detrimental factors have menaced the beekeeping industry of Mexico, such as the discovery of the parasitic mite *V. destructor* in 1992 (Chihu et al., 1992) and destructive climatic events, such as hurricanes (Guzman-Novoa et al., 2011). One decade later, in 2016, honey production statistics were similar to those of 2006. This pattern of decrease in honey production, followed by a posterior increase, is consistent with what has happened in Brazil and Venezuela. Initially, beekeepers experienced a loss of hives and production, but when they adopted new management practices and replaced or increased their lost colonies, honey production increased gradually (Hellmich and Rinderer, 1991; Rinderer and Collins, 1991; Gonçalves et al., 1991). Despite the partial increase of honey production in the last two decades, total honey production in Mexico has not been recovered to the levels prior to the Africanization of colonies. Selective breeding could contribute to increase honey production as Guzman-Novoa and Page (1999a,b) demonstrated, using local populations of AHBs in which they increased the frequency

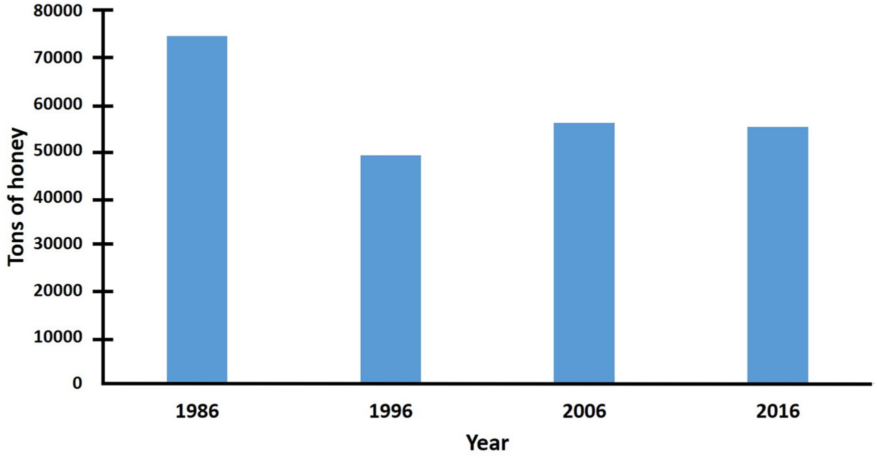

FIGURE 3 | Honey production in Mexico the year of the arrival of AHBs (1986), 10 (1996), 20 (2006), and 30 (2016) years later (data from Sader, 2020).

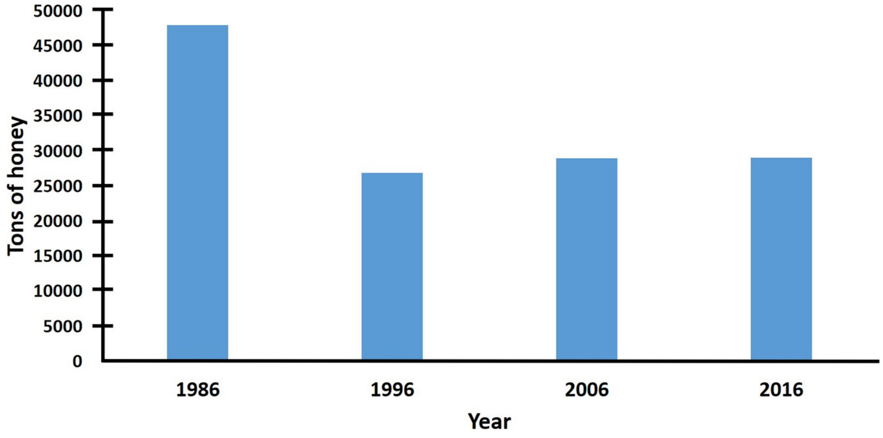

FIGURE 4 | Honey exports in Mexico the year of the arrival of AHBs (1986), 10 (1996), 20 (2006), and 30 (2016) years later (data from Sader, 2020).

of EHB genes and colony honey yields, in an Africanized region of Mexico.

Defensive Behavior

One of the most noticeable changes in honey bee colonies as a result of Africanization, is their increased defensiveness, which occurs at a rapid pace (Quezada-Euán and Paxton, 1999). AHBs react faster, with more individuals that pursue and sting, and their defensive responses last longer compared with EHB colonies (Stort, 1975a,b; Collins et al., 1982; Collins and Rinderer, 1991; Guzman-Novoa et al., 1994, 2002a,b; Giray et al., 2000; Uribe-Rubio et al., 2003; Breed et al., 2004). Numerous studies conducted in Mexico have contributed to better understand how environmental and genetic effects, as well as worker interactions, affect the defensive behavior of AHBs, and how this behavior has impacted society and beekeeping practices.

The defensive behavior of honey bees consists of several tasks, including guarding, pursuing and stinging (Breed et al., 2004). Guarding is a specialized behavior performed by less than 40% of the bees in a colony (Moore et al., 1987; Unger and Guzman-Novoa, 2012). Guards inspect incoming bees with their antennae at the colony entrance to distinguish foreign bees and other invertebrates from nestmates. They use olfactory cues to allow nestmates enter the colony and to reject foreign bees and other invertebrates (Moore et al., 1987). When guards cannot repel intruders, particularly vertebrates, they release alarm pheromones to recruit bees from the interior of the hive to pursue and sting intruders (Breed et al., 2004). Guards thus, play an important role in impeding the entrance of invertebrates to the colony and in recruiting nestmates to defend their colony against vertebrates.

To analyze genetic and environmental effects on the guarding behavior of AHBs and EHBs in Mexico, Hunt et al. (2003a) co-fostered known numbers of individually marked AHBs and EHBs of the same age in common colonies of both genotypes, and observed their guarding behavior. They found that AHBs guarded at a younger age, in higher numbers and during more days than EHBs. On average, AHBs guarded for 5 days and EHBs for 3 days. They concluded that genotypic, environmental and genotype x environmental effects significantly increased guarding bouts in the AHB genotype. In another study, Hunt et al. (2003b) reported that when bees of the two genotypes co-fostered in a common colony were exposed to alarm pheromone components (emulating a recruiting event by guard bees), AHBs responded in greater numbers.

Soldiers (the bees recruited by guards) comprise another group of defenders that pursue intruders to deter them from the colony and sting them if they do not go away (Breed et al., 2004). The association of guarding and soldiering tasks was demonstrated by Guzman-Novoa et al. (2004), who found a correlation between individual guarding and other components of defensive behavior, such as response to alarm pheromone, pursuing tendency, and stinging. Moreover, the distance and number of bees pursuing experimenters was the component that best separated gentle and defensive colonies. The mean number of pursuing AHBs at different distances was >81 times greater than that of pursuing EHBs (Guzman-Novoa et al., 2003).

Environmental effects and worker interactions also affect the defensive behavior of honey bees (Uribe-Rubio et al., 2003, 2008, 2013). It is likely that in Africanized areas, honey bee colonies are composed of multiple worker genotypes because queens mate with many drones of different genetic origins. To emulate these conditions, colonies were assembled by co-fostering EHBs and AHBs in common colonies in Tonatico, Mexico, and their stinging responses were quantified with leather patches presented at different times. During the first 10 s of the test, 81% of the bees that stung were AHBs, but from 10 to 30 s, AHBs and EHBs were equally likely to sting. However, when tested in their own environments, bees of two of the three EHB colonies used did not sting and did not pursue in any of the eight trials conducted, whereas all three AHB colonies did (Guzman-Novoa et al., 2004). These results suggest behavioral interactions among bees of different genotypes when they share a common nest. The more defensive type of bees may affect the response thresholds of less defensive bee genotypes, recruiting them to sting. Similarly, in a previous study, changes in propensity to sting in a gentle bee genotype were demonstrated. Co-fostered EHBs were more likely to sting in colonies containing hybrid (Africanized/European) bees than in their natal nests (Guzman-Novoa and Page, 2000).

In addition to environmental influences and worker interactions, genetic effects strongly influence the defensive behavior of AHBs (Hunt et al., 2007). Their intense defensive responses are highly heritable and apparently genetically dominant (Stort, 1975a,b; Collins and Rinderer, 1991; Guzman-Novoa and Page, 1994a; DeGrandi-Hoffman et al., 1998a; Hunt et al., 1998; Guzman-Novoa et al., 2002a), but when crossed with EHBs and analyzed by type of hybrid or backcross, it was found that AHB colonies in Mexico are strongly affected by paternal effects. Paternal effects could explain why in each of four sets of crosses, bees from hybrid colonies of Africanized paternity left more stings in leather patches than bees from hybrid colonies of European paternity (Guzman-Novoa et al., 2005). Although some degree of dominance was observed for high-defensive behavior in crosses involving European paternity, most of the dominance effects appear to be the result of paternal effects (**Figure 5**). Paternal effects on the defensive behavior of honey bees may explain in part the ecological success of AHBs. Defensive traits are beneficial to honey bee colonies in tropical environments because they help them reduce predation (Rinderer and Hellmich, 1991). Two potential mechanisms may explain a paternal effect for defensive behavior. First, interactions between European mitochondrial genes and African nuclear alleles could result in a more defensive response. Another explanation for the observed paternal effects is that imprinting mechanisms selectively reduce expression of maternal alleles or increase expression of paternal alleles. Imprinting usually is caused by methylation that silence particular alleles depending on the sex of the parent from which the alleles were inherited (Constancia et al., 1998).

Five putative quantitative trait loci (QTLs), including *sting-1-5* that influence honey bee defensive behavior were mapped in

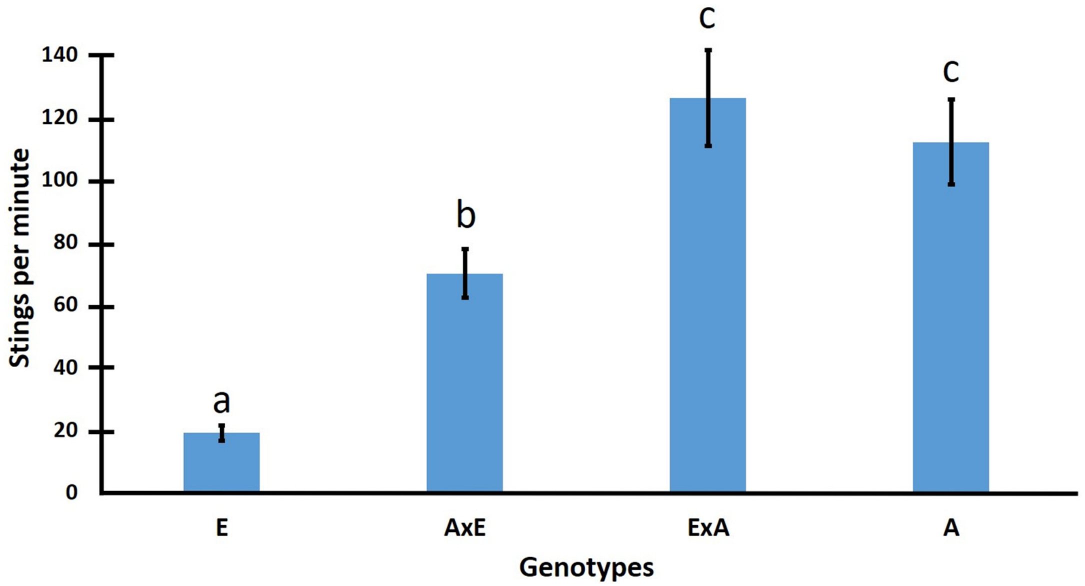

FIGURE 5 | Mean number of stings per minute on leather patches from four years of testing for colonies of four honey bee genotypes: E (European), AxE (F1 of European paternity) ExA (F1 of Africanized paternity), A (Africanized) (data from Guzman-Novoa et al., 2005).

a population of more than 300 backcrossed colonies tested in Mexico (Hunt et al., 1998). In another study, the marker genotypes near *sting-1* were associated with the tasks of guarding and fast stinging, thus confirming the effects of *sting-1* on defensive behavior (Guzman-Novoa et al., 2002b). Seven additional QTLs that regulate alarm pheromone production were mapped in Mexican AHB backcrossed colonies (Hunt et al., 1999). Other genes that regulate and have influenced the evolution of defensive responses in AHBs and EHBs were identified with microarray studies conducted in Mexico (Alaux et al., 2009; Ament et al., 2012). Recent work using pooled sequencing of AHBs identified 65 loci associated to defensive behavior from colonies that diverged in defensive responses, and showed that they contained African and European alleles that interacted (Harpur et al., 2020). By comparison, the apparently gentle AHB of Puerto Rico (Rivera-Marchand et al., 2008) differs in genetic structure from AHB populations in mainland United States, probably as a consequence of local selection and evolutionary processes on the island (Galindo-Cardona et al., 2013; Avalos et al., 2017; Acevedo-Gonzalez et al., 2019). Therefore, it is possible that different sets of genes may affect the defensive behavior of AHBs in different populations as a consequence of regional selection and interaction of African and European alleles.

Clearly, the numerous studies that have been conducted in Mexico have evidenced that the defensive behavior of AHB populations in the country varies, but it is clearly more pronounced than that of EHB populations and is affected by genetic effects, environmental effects, and complex interactions between worker bees in their nests. This higher level of defensiveness of AHBs has impacted human and animal health, and the beekeeping industry of Mexico.

Impact of Defensive Behavior

For many people in Mexico, the characterization of AHBs as “killer bees” added to public awareness of honey bee defensive behavior. Unfortunately, this characterization has created a fear-driven public climate in some regions of the country that is often antagonistic to populations of honey bees, regardless of actual hazards. This is because isolated stinging incidents and human fatalities cause a great impression in people and are not easily forgotten (Guzman-Novoa et al., 2011). Beekeepers are able to take precautions in the apiary and avoid serious stinging incidents, but it is not always possible to prevent interactions between the public and managed AHBs, particularly in dense populated areas. Incidents often occur when animals or humans walk nearby apiaries that have been recently manipulated by beekeepers, and are more common just before the main nectar flow because colonies are strongly populated (Guzman-Novoa et al., 2011).

The number people killed by honey bees in Mexico between 1988 and 2009 was 21.8 per year, or 0.23 per million people (Becerril-Ángeles et al., 2013), which is relatively insignificant. By comparison, scorpion stings cause 2.87 fatalities per million people (Celis et al., 2007). Therefore, the probability of being killed by honey bee stinging incidents in Mexico is about 12 times lower than being killed by scorpion stinging incidents, and it is 3,500 times less likely to die of bee stings than to die of diabetes (Becerril-Ángeles et al., 2013). However, despite the low probability of mortality, the arrival of AHBs temporarily increased the number of human fatalities between 1988 and 1993 and then progressively decreased in the following years until 2009, the last year of recorded fatalities by honey bee stings in Mexico (**Figure 6**). From 1988 to 2009 there were 480 fatality cases, and more than 70% of them were people 50 years

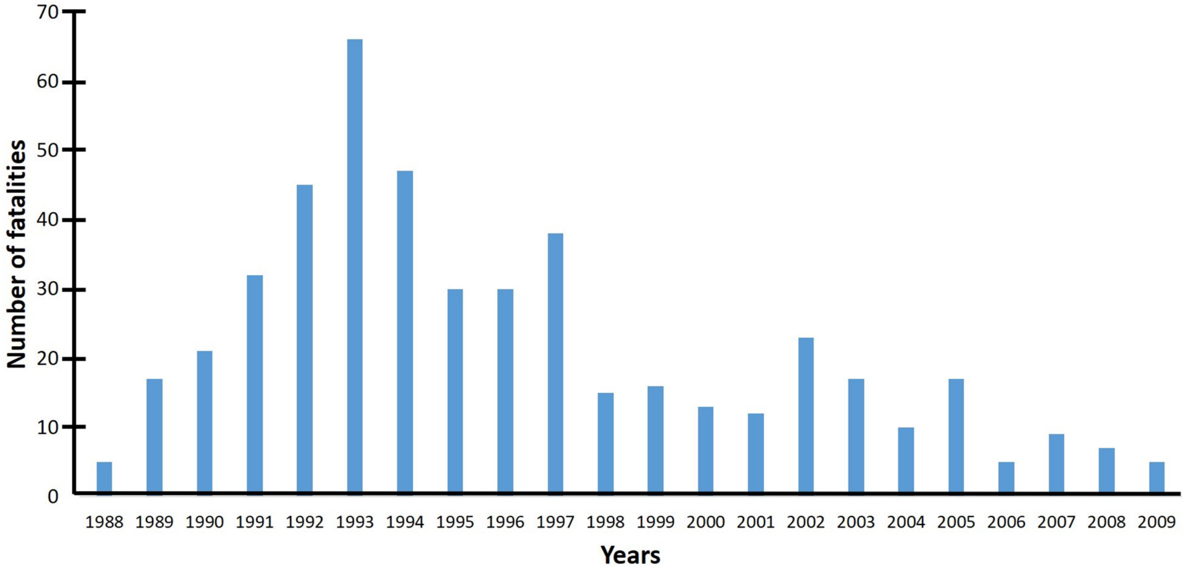

FIGURE 6 | Number of death people from honey bee stings in Mexico between 1988 and 2009 (data from Becerril-Ángeles et al., 2013). By comparison, the probability of being killed by honey bee stinging incidents in Mexico is about 12 times lower than the probability of being killed by scorpion stinging incidents.

of age or older (Becerril-Ángeles et al., 2013). It is believed that the gradual decrease in the number of human fatalities between 1994 and 2009 was probably due to the sum of several factors, including more awareness in the population about the danger that approaching honey bee nests and hives represents, the elimination of more than 100,000 swarms annually by fire fighters (Becerril-Ángeles et al., 2013), the relocation of apiaries away from urban areas and main roads by beekeepers, and a higher requeening rate of honey bee colonies with gentler stock (Guzman-Novoa et al., 2011).

Management Practices and Breeding

A consequence of working with more defensive bees after the arrival of AHBs is that some beekeepers have abandoned the activity, or in the case of commercial beekeepers, reduced their colony numbers, because finding locations suitable for apiaries has become more difficult. Landowners who have fields suitable for beekeeping do not easily accept the establishment of hives within their property because they want to avoid problems related to stinging incidents (Guzman-Novoa et al., 2011). In fact, large operations of more than 5,000 hives mainly dedicated to honey production went out of business. Examples of this were "Miel Carlota," "Acapulco Miel," and "Veramiel" companies with more than 40,000, 20,000, and 10,000 colonies, respectively. The only large beekeeping operations remaining in Mexico after the colonization of AHBs of most beekeeping regions of the country, are those mainly dedicated to rent their colonies for crop pollination, because hive rental fees are high enough to provide profits above hive management costs (Magaña-Magaña et al., 2016). Those operations use bee stocks selected for reduced defensive behavior (Guzman-Novoa et al., 2011).

Although large commercial beekeeping operations have decreased since the arrival of AHBs, the number of small-scale and sideline beekeepers owning between 20 and 500 hives has increased, and it is expected that this trend continue (Guzman-Novoa et al., 2011). These beekeepers do not hire labor because they manage their own colonies or receive help from family members and relatives, with which labor costs and the difficulty of finding beekeeping employees do not affect them as much as large commercial companies are affected. Furthermore, their apiaries are normally located on sites close to their place of residence. Therefore, they do not have the transportation costs or the difficulties associated with finding suitable locations to place their apiaries as large commercial beekeeping companies have experienced.

Mexican beekeepers have adapted to the "new" bees by changing their management practices to better deal with some of the pronounced behaviors of AHBs, particularly their extreme defensive, swarming, and absconding behaviors. Small-scale beekeepers have adopted fewer changes than commercial beekeepers, but all have changed their management practices to some extent. According to Guzman-Novoa et al. (2011), among the practices most commonly adopted by beekeepers to manage honey bee colonies in Africanized areas of Mexico are: (1) the use of better protective equipment such as coveralls and gloves, (2) the requeening of defensive colonies with queens from gentler stock, (3) the relocation of apiaries to isolated areas to reduce the probability of stinging incidents involving humans and livestock, (4) the feeding of colonies during dearth periods to decrease colony loses due to absconding behavior, and (5) the more frequent swarming control and honey harvests.

The above practices have improved the management and productivity of honey bee colonies in Africanized areas. Requeening is an important measure because queens in tropical environments are frequently superseded. In a study conducted in an Africanized area of Mexico, the queens introduced into more than 350 colonies were monitored for 1 year. After 6 and 12 months, only 61% and less than 30% of the introduced queens, respectively, were found in the colonies (Guzman-Novoa et al., 1998). Therefore, the requeening of colonies is recommended as a minimum once a year (Guzman-Novoa et al., 2011). Fortunately, queen acceptance rates in AHB colonies are as high as those in EHB colonies (Guzman-Novoa et al., 1997). Additionally, the relocation of apiaries has decreased the number of stinging incidents (Becerril-Ángeles et al., 2013), and the last two measures have resulted in increased honey yields (Guzman-Novoa et al., 2011).

Previous studies have demonstrated that if the Africanization level of colonies is 25% or lower, bees of these colonies do not differ in stinging behavior with EHB colonies (Hellmich, 1991; Guzman-Novoa and Page, 1993, 1999a,b). Therefore, selective bee breeding could increase honey yields and decrease the defensive behavior of AHB populations by decreasing the Africanization level of colonies. Guzman-Novoa and Page (1999b) conducted a long-term selective breeding program in an open honey bee population of more than 3,000 colonies located in an Africanized area of Mexico. This program is the largest selective breeding program so far conducted in Africanized bee regions. After 5 years of selection, honey yields increased 16%, stinging behavior decreased 54%, and the length of worker wings increased >1%. Additionally, the percentage of colonies having bees with African mtDNA decreased from 28% before selection to 7% after four generations of selection. These results suggest that the level of Africanization in the selected population was reduced to some degree over time, and demonstrate that it is possible to breed gentler and more productive bees in Africanized areas without the use of instrumental insemination of queen bees. Programs like this, stimulated the implementation of other selective breeding programs for low defensive behavior (Esquivel-Rojas et al., 2015) and also queen breeders who selectively breed honey bee stock for higher honey yields and lower defensiveness, have considerably increased the number of queens they rear and sell. In fact, according to the Mexican association of queen breeders, the number of queens reared tripled in 30 years (Guzman-Novoa et al., 2011). In the Yucatan peninsula, selection of AHBs has also resulted in increased honey yields, disease resistance and lower defensiveness. Those promising results suggest that selection of AHBs, rather than the introduction of European stock seems to be the best option for tropical areas (Quezada-Euán et al., 2008; Zárate et al., 2008).

CONCLUSION AND FURTHER DIRECTIONS

The Africanization of the honey bees of Mexico has resulted in extensive hybridization strongly driven by climatic conditions. As a result, AHB colonies are amongst the most genetically diverse *A. mellifera* yet recorded (Tarpy et al., 2010; Harpur et al., 2012, 2020). Notably, AHBs do not resemble anymore their original ancestors, they are genetically and biologically distinct, and might be considered a separate *A. mellifera* lineage well adapted to the Neotropics. Presently, the managed colonies of honey bees in Mexico show two contrasting patterns, one mainly composed by AHBs in the tropical beekeeping regions of the Yucatan peninsula, the Gulf of Mexico's coast and the Pacific Coast, and another where EHBs predominate, in the temperate beekeeping regions of the high plateau and the North of the country. However, little is known about the composition of feral populations elsewhere in the country. Future studies of Mexican honey bees involving high-density molecular markers should be useful in revealing their population genetic structure, especially in lesser studied regions. No information exists on the patterns of introgression at nuclear loci. Thus, the level of selection that has occurred for African and European-derived genes in different regions as well as the genetic stability of such populations is still unclear. Moreover, possible AHBs-EHBs clines and hybrid zones may exist in Mexico, but this has not been studied extensively (see Kraus et al., 2007).

The Africanization of honey bees has resulted in lower honey yields per colony in temperate climates of Mexico, but how it has affected honey yields in the tropics is unknown. Therefore, side by side comparative studies of honey production and about the underlying factors that affect this trait between AHB and EHB colonies in the tropics are warranted. The defensive behavior of bees in Mexico has increased, but the impact of this behavior on stinging incidents involving humans has been relatively insignificant. A new generation of beekeepers emerged and learned how to manage AHBs to reduce their impact and to make them more productive. A corollary is that breeding programs in Mexico and elsewhere in the Neotropics, may benefit from using honey bees that are already present and better adapted to their respective tropical and temperate zones. These populations have been largely confronted to various environmental conditions and in the process are better adapted compared with foreign stock (Domínguez-Ayala et al., 2016). This is particularly relevant considering the low frequency of massive colony losses in Mexico and Latin America due to Colony Collapse Disorder and diseases, compared to what occurs in the United States and Europe (Vandame and Palacio, 2010). Moreover, the discovery and use of nuclear markers for assisted selection, should accelerate the breeding of gentler, more productive and disease resistant honey bees.

In comparison with the economic impact, in Mexico (as well as all over the Americas), the assessment of ecological impacts as a result of the Africanization of honey bees is meager. Although some positive effects have occurred, such as the increase of pollination services to some crops and the concomitant impulse of stingless beekeeping, the large-scale impact of AHBs on native ecosystems as well as on specialized bee-plant interactions is virtually unknown. Further studies should focus on ways that help maintaining an equilibrium between AHBs and native species. Better characterization of pollination mutualisms in wild and managed systems is also crucial to ensure habitat stability and agricultural production (Roubik, 2000). The evidence suggests that under limited food resources, competition can become more intense, possibly leading to limits where the systems can no longer adapt (Roubik, 2009). Therefore, one way to protect such equilibrium is by preserving and, if possible, increasing natural areas with rich floral resources, especially in regions where beekeeping is intensively practiced. Recent evidence suggests that AHBs may tolerate high levels of heat stress (Medina et al., 2018; Medina et al., 2020; Poot-Baez et al., 2020), a fact that should be considered for the sustainability of beekeeping under the threat of climate change but, comparative studies with EHBs are still needed. Research could help to better understand the basis of biological adaptations and behaviors of AHBs to develop technologies aimed at making them more productive and less defensive. AHBs are in the Americas to stay and the challenge is to make sustainable use of them while preserving the ecosystems in which they now thrive.

AUTHOR CONTRIBUTIONS

EG-N, NM, JT-G, JM-M, FC-E, and JQ-E contributed to the original idea and structure of this manuscript. CM-F and AC-B collected the data. JQ-E and JM-M prepared the figures. AD and NM formatted the manuscript. All authors contributed to write and revise the final version of the manuscript.

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REFERENCES

Acevedo-Gonzalez, J. P., Galindo-Cardona, A., Avalos, A., Whitfield, C. W., Rodriguez, D. M., Uribe-Rubio, J. L., et al. (2019). Colonization history and population differentiation of the honey bees (*Apis mellifera* L.) *in Puerto Rico*. *Ecol. Evol.* 9, 1–8. doi: 10.1002/ece3.5330

Alaux, C., Sinha, S., Hasadsri, L., Hunt, G. J., Guzman-Novoa, E., DeGrandi-Hoffman, G., et al. (2009). Honey bee aggression supports a link between gene regulation and behavioral evolution. *PNAS* 106, 15400–15405. doi: 10.1073/pnas.0907043106

Ament, S. A., Blatti, C., Alaux, C., Wheeler, M. M., Toth, A. L., Le Conte, Y., et al. (2012). New meta-analysis tools reveal common transcriptional regulatory basis for multiple determinants of behavior. *PNAS* 109, 1801–1810. doi: 10.1073/pnas.1205283109

Avalos, A., Pan, H., Li, C., Acevedo-Gonzalez, J. P., Rendon, G., Fields, C. J., et al. (2017). A soft selective sweep during rapid evolution of gentle behaviour in an Africanized honeybee. *Nat. Commun.* 8:1550. doi: 10.1038/s41467-017-01800-0

Becerra-Guzmán, F., Guzman-Novoa, E., Correa-Benítez, A., and Zozaya, A. (2005). Length of life, age at first foraging, and foraging life of Africanized and European Honeybee (*Apis mellifera* L.) workers during conditions of resource abundance. *J. Apic. Res.* 44, 151–156. doi: 10.1080/00218839.2005.11101170

Becerril-Ángeles, M., Núñez-Velázques, M., and Arias-Martínez, M. I. (2013). Mortalidad en México relacionada con picaduras de abeja de 1988 a 2009. *Rev. Alergia Mex.* 60, 58–62.

Breed, M. D., Guzman-Novoa, E., and Hunt, G. J. (2004). Defensive behavior of honey bees: organization, genetics and comparisons with other bees. *Annu. Rev. Entomol.* 49, 271–298. doi: 10.1146/annurev.ento.49.061802.123155

Caro, A., Moo-Valle, H., Alfaro, R., and Quezada-Euán, J. J. G. (2017). Pollination services of Africanized honey bees and native *Melipona beecheii* to buzz-pollinated annatto (*Bixa orellana* L.) *in the neotropics*. *Agric. For. Entomol.* 19:274. doi: 10.1111/afe.12206

Caron, D. M. (2001). *Africanized Bees in the Americas*. Medina OH: A.I. Root Co.

Celis, A., Gaxiola-Robles, R., Sevilla-Godínez, E., Orozco-Valerio, M. J., and Armas, J. (2007). Tendencia de la mortalidad por picaduras de alacrán en México, 1979-2003. *Rev. Panam. Salud Publ.* 21, 373–380.

Chihu, A. D., Rojas, L. M., and Rodríguez, S. R. (1992). "Primer reporte en México del ácaro *Varroa jacobsoni*, causante de la varroasis de la abeja melífera (*Apis mellifera* L.)," in *Proceedings of the Memorias VI Sem. Am. Apic*, Oaxtepéc, México, 9–11.

Clarke, K. E., Rinderer, T. E., Franck, P., Quezada-Euán, J. J. G., and Oldroyd, B. P. (2002). The Africanization of honeybees (*Apis mellifera* L.) *of the Yucatan: a study of a massive hybridization event across time*. *Evolution* 56, 1462–1474. doi: 10.1111/j.0014-3820.2002.tb01458.x

Collins, A. M., and Rinderer, T. E. (1991). "Genetics of defensive behavior, I," in *The "African" honey bee*, eds M. Spivak, D. J. C. Fletcher, and M. D. Breed (Boulder, CO: Westview Press), 309–328. doi: 10.1201/9780429308741-16

Collins, A. M., Rinderer, T. E., Harbo, J. R., and Bolten, A. B. (1982). Colony defense by Africanized and European honey bees. *Science* 218, 72–74. doi: 10.1126/science.218.4567.72

Constancia, M., Pickard, B., Kelsey, G., and Reik, W. (1998). Imprinting mechanisms. *Genome Res.* 8, 881–900.

Daly, H. V., and Balling, S. S. (1978). Identification of Africanized honey bees in the western hemisphere by discriminant analysis. *J. Kans. Entomol. Soc.* 51, 857–869.

Danka, R. G., Hellmich, R. L., and Rinderer, T. E. (1992). Nest usurpation, supersedure and colony failure contribute to Africanization of commercially managed European honey bees in Venezuela. *J. Apic. Res.* 31, 119–123. doi: 10.1080/00218839.1992.11101272

Danka, R. G., Hellmich, R. L., Rinderer, T. E., and Collins, A. M. (1987). Diet-selection ecology of tropically and temperately adapted honey bees. *Anim. Behav.* 35, 1858–1863. doi: 10.1016/s0003-3472(87)80078-7

De Souza, F. S., Kevill, J. L., Correia-Oliveira, M. E., de Carvalho, C. A. L., and Martin, S. J. (2019). Occurrence of deformed wing virus variants in the stingless bee *Melipona subnitida* and honey bee *Apis mellifera* populations in Brazil. *J. Gen. Virol.* 100, 289–294. doi: 10.1099/jgv.0.001206

DeGrandi-Hoffman, G., Collins, A. M., Martin, J. H., Schmidt, J. O., and Spangler, H. G. (1998a). Nest defense behavior in colonies from crosses between Africanized and European honey bees (*Apis mellifera* L.) *(Hymenoptera: Apidae)*. *J. Insect. Behav.* 11, 37–45.

DeGrandi-Hoffman, G., Watkins, J. C., Collins, A. M., Loper, G. M., Martin, J. H., and Arias, M. C. (1998b). Queen developmental time as a factor in the Africanization of European honey bee (Hymenoptera: Apidae) populations. *Ann. Entomol. Soc. Am.* 91, 52–58. doi: 10.1093/aesa/91.1.52

Delgado-Carrillo, O., Martén-Rodríguez, S., Ashworth, L., Aguilar, R., Lopezaraiza-Mikel, M., and Quesada, M. (2018). Temporal variation in pollination services to *Cucurbita moschata* is determined by bee gender and diversity. *Ecosphere* 9, 1–16.

Do Carmo, R. M., Franceschinelli, E. V., and de Silveira, F. A. (2004). Introduced honeybees (*Apis mellifera*) reduce pollination success without affecting the floral resource taken by native pollinators. *Biotropica* 36, 371–376. doi: 10.1111/j.1744-7429.2004.tb00329.x

Domínguez-Ayala, R., Moo-Valle, H., May-Itzá, W. J., Medina-Peralta, S., and Quezada-Euán, J. J. G. (2016). Stock composition of northern Neotropical honey bees: mitotype and morphotype diversity in Mexico

(Hymenoptera: Apidae). *Apidologie* 47, 642–652. doi: 10.1007/s13592-015-0414-6

Efsthation, C. A., and Kern, W. H. Jr. (2016). A push-pull protocol to reduce colonization of bird nest boxes by honey bees. *J. Vis. Exp.* 115:53950. doi: 10.3791/53950

Esquivel-Rojas, S., Macías-Macías, J. O., Tapia-González, J. M., Contreras-Escareño, F., De León-Mantecón, M. J., and Silva-Contreras, A. (2015). Selección de abejas (*Apis mellifera* L) con baja defensividad y su relación con el medio ambiente en Jalisco, México. *Abanico Veterinario* 5, 44–50.

Fewell, J. H., and Bertram, S. M. (2002). Evidence for genetic variation in worker task performance by African and European honey bees. *Behav. Ecol. Sociobiol.* 52, 318–325. doi: 10.1007/s00265-002-0501-3

Fierro, M. M., Barraza, A., Maki, D. L., and Moffet, J. O. (1987). The effects of the first year of Africanization of honey bee populations in Chiapas. *Mexico. Am. Bee. J.* 127:845.

Fleites-Ayil, F. A., Quezada-Euán, J. J. G., and Medina-Medina, L. A. (2018). Onset of foraging and lifespan of Africanized honey bees (*Apis mellifera*) infected with different levels of *Nosema ceranae* spores in Neotropical Mexico. *Apidologie* 49, 781–788. doi: 10.1007/s13592-018-0602-2

Galindo-Cardona, A., Acevedo-Gonzalez, J. P., Rivera-Marchand, B., and Giray, T. (2013). Genetic structure of the gentle Africanized honey bee population (gAHB) in Puerto Rico. *BMC Genet.* 14:65. doi: 10.1186/1471-2156-14-65

Giray, T., Guzman-Novoa, E., Aron, C. W., Zelinsky, B., Fahrbach, S. E., and Robinson, G. E. (2000). Genetic variation in worker temporal polyethism and colony defensiveness in the honey bee, *Apis mellifera*. *Behav. Ecol.* 11, 44–55. doi: 10.1093/beheco/11.1.44

Gonçalves, L. S., Stort, A. C., and De Jong, D. (1991). "Beekeeping in Brazil," in *The "African" Honey Bee*, eds M. Spivak, D. J. C. Fletcher, and M. D. Breed (Boulder, CO: Westview), 359–372. doi: 10.1201/9780429308741-18

Goulson, D. (2003). Effects of introduced bees on native ecosystems. *Annu. Rev. Ecol. Evol. Syst.* 34, 1–26. doi: 10.1146/annurev.ecolsys.34.011802.132355

Guzman-Novoa, E. (1996). "La Apicultura en México y Centro América," in *Proceedings of the Memorias del V Congreso Ibero Latinoamericano de Apicultura 1996*, (Mercedes: Intendenc. Municip. Soriano, Central Apic. Coop. Calmer), 14–17.

Guzman-Novoa, E. (2004). Impacto de la Africanización de las abejas en México. *Imagen Veterinaria* 4, 22–25.

Guzman-Novoa, E., Correa-Benítez, A., Guzman, G., and Espinoza-Montaño, L. G. (2011). Colonization, impact and control of Africanized honey bees in Mexico. *Veterinaria Mex.* 42, 149–178.

Guzman-Novoa, E., Eccles, L., Calvete, Y., McGowan, J., Kelly, P. G., and Correa-Benítez, A. (2010). *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario. Canada. *Apidologie* 41, 443–450. doi: 10.1051/apido/2009076

Guzman-Novoa, E., and Gary, N. E. (1993). Genotypic variability of components of foraging behavior in honey bees (Hymenoptera: Apidae). *Ann. Entomol. Soc. Am.* 83, 1152–1154.

Guzman-Novoa, E., Hamiduzzaman, M. M., Anguiano-Baez, R., Correa-Benítez, A., Castañeda-Cervantes, E., and Arnold, N. I. (2015). First detection of honey bee viruses in stingless bees in North America. *J. Apic. Res.* 54, 93–95. doi: 10.1080/00218839.2015.1100154

Guzman-Novoa, E., Hunt, G. J., Page, R. E. Jr., and Fondrk, M. K. (2002a). Genetic correlations among honey bee (Hymenoptera: Apidae) behavioral characteristics and wing length. *Ann. Entomol. Soc. Am.* 95, 402–406. doi: 10.1603/0013-8746(2002)095[0402:gcahbh]2.0.co;2

Guzman-Novoa, E., Hunt, G. J., Page, R. E. Jr., Uribe-Rubio, J. L., Prieto-Merlos, D., and Becerra-Guzmán, F. (2005). Paternal effects on the defensive behavior of honeybees. *J. Hered.* 96, 376–380. doi: 10.1093/jhered/esi038

Guzman-Novoa, E., Hunt, G. J., Uribe-Rubio, J. L., and Prieto-Merlos, D. (2004). Genotypic effects of honey bee (*Apis mellifera*) defensive behavior at the individual and colony levels: the relationship of guarding, pursuing and stinging. *Apidologie* 35, 15–24. doi: 10.1051/apido:2003061

Guzman-Novoa, E., Hunt, G. J., Uribe-Rubio, J. L., Smith, C., and Arechavaleta-Velasco, M. E. (2002b). Confirmation of QTL effects and evidence of genetic dominance of honey bee defensive behavior: results of colony and individual behavioral assays. *Behav. Genet.* 32, 95–102.

Guzman-Novoa, E., and Page, R. E. Jr. (1999a). "Breeding honey bees in Africanized areas," in *Apiculture for the 21st Century*, eds R. Hoopingarner and L. Connor (Cheshire, CT: Wicwas Press), 12–14.

Guzman-Novoa, E., and Page, R. E. Jr. (1999b). Selective breeding of honey bees (Hymenoptera: Apidae) in Africanized areas. *J. Econ. Entomol.* 92, 521–525. doi: 10.1093/jee/92.3.521

Guzman-Novoa, E., Page, R. E. Jr., and Correa-Benítez, A. (1997). Introduction and acceptance of European queens in Africanized and European honey bee (*Apis mellifera* L.) *colonies*. *Am. Bee J.* 137, 667–668.

Guzman-Novoa, E., Page, R. E. Jr., and Fondrk, M. K. (1994). Morphometric techniques do not detect intermediate and low levels of Africanization in honey bee (Hymenoptera: Apidae) colonies. *Ann. Entomol. Soc. Am.* 87, 507–515. doi: 10.1093/aesa/87.5.507

Guzman-Novoa, E., Page, R. E. Jr., and Prieto-Merlos, D. (1998). Queen introduction, acceptance, and survival in honey bee (Hymenoptera: Apidae) colonies of a tropical. Africanized region. *J. Econ. Entomol.* 91, 1290–1294. doi: 10.1093/jee/91.6.1290

Guzman-Novoa, E., and Page, R. E. (1993). Backcrossing Africanized honey bee queens to European drones reduces colony defensive behavior. *Ann. Entomol. Soc. Am.* 86, 352–355. doi: 10.1093/aesa/86.3.352

Guzman-Novoa, E., and Page, R. E. (1994a). Genetic dominance and worker interactions affect honey bee colony defense. *Behav. Ecol.* 5, 91–97. doi: 10.1093/beheco/5.1.91

Guzman-Novoa, E., and Page, R. E. (1994b). The impact of Africanized bees on Mexican beekeeping. *Am. Bee. J.* 134, 101–106.

Guzman-Novoa, E., and Page, R. E., Jr. (2000). Pollen collection and foraging force by European and European x Africanized hybrid honey bees (Hymenoptera: Apidae) in mixed genotype colonies are similar. *Ann. Entomol. Soc. Am*. 93, 141–144.

Guzman-Novoa, E., Prieto-Merlos, D., Uribe-Rubio, J. L., and Hunt, G. J. (2003). Relative reliability of four field assays to test defensive behaviour of honey bees (*Apis mellifera*). *J. Apic. Res.* 42, 42–46. doi: 10.1080/00218839.2003.11101088

Guzman-Novoa, E., and Uribe-Rubio, J. L. (2004). Honey production by European. *Africanized and hybrid honey bee (Apis mellifera*) colonies in Mexico. *Am. Bee. J.* 144, 318–320.

Guzman-Novoa, E., Vandame, R., and Arechavaleta-Velasco, M. E. (1999). Susceptibility of European and Africanized honey bees (*Apis mellifera* L.) *to Varroa jacobsoni Oud. in Mexico*. *Apidologie* 30, 173–182. doi: 10.1051/apido:19990207

Hall, H. G., and Muralidharan, K. (1989). Evidence from mitochondrial DNA that African honey bees spread as continuous maternal lineages. *Nature* 339, 211–213. doi: 10.1038/339211a0

Hamiduzzaman, M. M., Guzman-Novoa, E., Goodwin, P. H., Reyes-Quintana, M., Koleoglu, G., Correa-Benítez, A., et al. (2015). Differential responses of Africanized and European honey bees (*Apis mellifera*) to viral replication following mechanical transmission or *Varroa destructor* parasitism. *J. Invertebr. Pathol.* 126, 12–20. doi: 10.1016/j.jip.2014.12.004

Harpur, B. A., Kadri, S. M., Orsi, R. O., Whitfield, C. W., and Zayed, A. (2020). Defense response in Brazilian honey bees (*Apis mellifera scutellata* x spp.) is underpinned by complex patterns of admixture. *Genom. Biol. Evol.* 12, 1367–1377. doi: 10.1093/gbe/evaa128

Harpur, B. A., Minae, S., Kent, C. F., and Zayed, A. (2012). Management increases genetic diversity of honey bees via admixture. *Mol. Ecol.* 21, 4414–4421. doi: 10.1111/j.1365-294x.2012.05614.x

Harrison, J. F., and Hall, H. G. (1993). African–European honeybee hybrids have low intermediate metabolic capacities. *Nature* 363, 258–259. doi: 10.1038/363258a0

Hellmich, R. L. (1991). "Continuing commercial queen production after the arrival of Africanized honey bees," in *The "African" Honey Bee*, eds M. Spivak, D. J. C. Fletcher, and M. D. Breed (Boulder, CO: Westview), 187–197. doi: 10.1201/9780429308741-9

Hellmich, R. L., and Rinderer, T. E. (1991). "Beekeeping in Venezuela," in *The "African" Honey Bee*, eds M. Spivak, D. J. C. Fletcher, and M. D. Breed (Boulder, CO: Westview), 399–411. doi: 10.1201/9780429308741-20

Hunt, G. J., Amdam, G. V., Schlipalius, D., Emore, C., Sardesai, N., Williams, C. E., et al. (2007). Behavioral genomics of honeybee foraging and nest defense. *Naturwissenschaften* 94, 247–267. doi: 10.1007/s00114-006-0183-1

Hunt, G. J., Collins, A. M., Rivera, R., Page, R. E. Jr., and Guzman-Novoa, E. (1999). Quantitative trait loci influencing honey bee alarm pheromone levels. *J. Hered.* 90, 585–589. doi: 10.1093/jhered/90.5.585

Hunt, G. J., Guzman-Novoa, E., Fondrk, M. K., and Page, R. E. Jr. (1998). Quantitative trait loci for honey bee stinging behavior and body size. *Genetics* 148, 1203–1213.

Hunt, G. J., Guzman-Novoa, E., Uribe-Rubio, J. L., and Prieto-Merlos, D. (2003a). Genotype by environment interactions in honeybee guarding behavior. *Anim. Behav.* 66, 459–467. doi: 10.1006/anbe.2003.2253

Hunt, G. J., Wood, K. V., Guzman-Novoa, E., Lee, H. D., Rothwell, A. P., and Bonham, C. C. (2003b). Discovery of 3-methyl-2-buten-1-yl acetate, a new alarm component in the sting apparatus of Africanized honey bees. *J. Chem. Ecol.* 29, 451–461.

Kerr, W. E. (1967). The history of the introduction of African Bees to Brazil. S. *Afr. Bee. J.* 39, 3–5.

Kraus, F. B., Franck, P., and Vandame, R. (2007). Asymmetric introgression of African genes in honeybee populations (*Apis mellifera* L.) *in Central Mexico*. *Heredity* 99, 233–240. doi: 10.1038/sj.hdy.6800988

Labougle, J. M., Mancera, M., and Taylor, O. R. (1989). Morphometric and ellectrophoretic study of the African honey bee in southern Mexico. *Am. Bee. J.* 129, 817.

Labougle, J. M., and Zozaya, A. (1986). La apicultura en México. *Cien. Desarrollo* 69, 17–36.

Landaverde-González, P., Quezada-Euán, J. J. G., Theodorou, P., Murray, T. E., Husemann, M., Ayala, R., et al. (2017). Sweat bees on hot chillies: provision of pollination services by native bees in traditional slash-and-burn agriculture in the Yucatan peninsula of tropical Mexico. *J. Appl. Ecol.* 54, 1814–1824. doi: 10.1111/1365-2664.12860

Macías-Macías, J. O., Tapia-Rivera, J. C., De la Mora, A., Tapia-González, J. M., Contreras-Escareño, F., Petukhova, T., et al. (2020). Nosema ceranae causes cellular immunosuppression and interacts with thiamethoxam to increase mortality in the stingless bee *Melipona colimana*. *Sci. Rep.* 10:17021.

Macías-Macías, O., Chuc, P., Ancona-Xiu, P., Cauich, O., and Quezada-Euán, J. J. G. (2009). Contribution of native bees and Africanized honeybees (Hymenoptera: Apoidea) to Solanaceae crop pollination in tropical Mexico. *J. Appl. Entomol.* 133, 456–465. doi: 10.1111/j.1439-0418.2009.01399.x

Magaña-Magaña, M. A., Tavera-Cortés, M. E., Salazar-Barrientos, L. L., and Sanginés-García, J. R. (2016). Productividad de la apicultura en México y su impacto sobre la rentabilidad. *Rev. Mexicana Cienc. Agrícol.* 7, 1103–1115. doi: 10.29312/remexca.v7i5.235

Martin, S. J., and Medina-Medina, L. (2004). Africanized bees have a unique tolerance to Varroa mites. *Trends. Parasitol.* 20, 112–114. doi: 10.1016/j.pt.2004.01.001

McMahon, D. P., Fürst, M. A., Caspar, J., Theodorou, P., Brown, M. J. F., and Paxton, R. J. (2015). A sting in the spit: widespread cross-infection of multiple RNA viruses across wild and managed bees. *J. Anim. Ecol.* 84, 615–624. doi: 10.1111/1365-2656.12345

Medina, R. G., Paxton, R. J., De Luna, E., Fleites-Ayil, F., Medina-Medina, L. A., and Quezada-Euán, J. J. G. (2018). Developmental stability, age at onset of foraging and longevity of Africanized honey bees (*Apis mellifera* L.) under heat stress (Hymenoptera: Apidae). *J. Therm. Biol.* 74, 214–225. doi: 10.1016/j.jtherbio.2018.04.003

Medina, R. G., Paxton, R. J., Hernández-Sotomayor, S. M. T., Pech-Jimenez, C., Medina-Medina, L. A., and Quezada-Euán, J. J. G. (2020). Heat stress during development affects immunocompetence in workers, queens and drones of Africanized honey bees (*Apis mellifera* L.) *(Hymenoptera: Apidae)*. *J. Therm. Biol.* 89:102541. doi: 10.1016/j.jtherbio.2020.102541

Medina-Flores, C. A., Guzman-Novoa, E., Hamiduzzaman, H. H., Aguilera-Soto, J., and Lopez-Carlos, M. A. (2015). Africanization of honey bees (*Apis mellifera*) in three climatic regions of northern Mexico. *Veter. Mex.* 2, 1–9. doi: 10.17221/7240-vetmed

Medina-Flores, C. A., Guzman-Novoa, E., Hamiduzzaman, M. M., Aréchiga-Flores, C. F., and López-Carlos, M. A. (2014). Africanized honey bees (*Apis mellifera*) have low infestation levels of the mite *Varroa destructor* in different ecological regions in Mexico. *Genet. Mol. Res.* 13, 7282–7293. doi: 10.4238/2014.february.21.10

Moffett, J. O., Maki, D. L., Andre, T., and Fierro, M. M. (1987). The Africanized bee in Chiapas. *Mexico. Am. Bee. J.* 127, 517–520.

Moore, A. J., Breed, M. D., and Moor, M. J. (1987). The guard honey bee: ontogeny and behavioral variability of workers performing a specialized task. *Anim. Behav.* 35, 1159–1167. doi: 10.1016/s0003-3472(87)80172-0

Morfin, N., Gashout, H. A., Macías-Macías, J. O., De la Mora, A., Tapia-Rivera, J. C., Tapia-González, J. M., et al. (2020). Detection, replication and quantification of deformed wing virus-A, deformed wing virus-B, and black queen cell virus in the endemic stingless bee, *Melipona colimana*, from Jalisco, Mexico. *Int. J. Trop. Insect. Sci.* doi: 10.1007/s42690-020-00320-7

Neuman, L. S. (2001). *Evaluación del comportamiento de pecoreo en tres genotipos de abejas melíferas (Europeas, híbridas y Africanizadas)*. DVM Thesis, National Autonomous University of Mexico, Mexico.

Nogueira-Neto, P. (1964). The spread of a fierce African bee in Brazil. *Bee World* 45, 119–121. doi: 10.1080/0005772x.1964.11097060

Page, R. E. (1989). Neotropical African bees. *Nature* 339, 181–182. doi: 10.1038/339181b0

Pérez-Balam, J., Quezada-Euán, J. J. G., Alfaro-Bates, R., Medina, S., McKendrick, L., Soro, A., et al. (2012). The contribution of honey bees, wasps and flies to avocado (*Persea americana*) pollination in southern Mexico. *J. Pollinat. Ecol.* 8, 42–47.

Pesante, D. G., Rinderer, T. E., and Collins, A. M. (1987). Differential nectar foraging by Africanized and European honeybees in the neotropics. *J. Apic. Res.* 26, 210–216. doi: 10.1080/00218839.1987.11100762

Poot-Baez, V., Medina-Hernández, R., Medina-Peralta, S., and Quezada-Euán, J. J. G. (2020). Intranidal temperature and body size of Africanized honey bees under heat waves (Hymenoptera: Apidae). *Apidologie* 51, 382–390. doi: 10.1007/s13592-019-00725-5

Portman, Z. M., Tepedino, V. J., Tripodi, A. D., Szalanski, A. L., and Durham, S. L. (2018). Local extinction of a rare plant pollinator in Southern Utah (USA) associated with invasion by Africanized honey bees. *Biol. Invasions* 20, 593–606. doi: 10.1007/s10530-017-1559-1

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., and Kunin, W. E. (2010). Global pollinator declines: trends, impacts, and drivers. *Trends. Ecol. Evol.* 25, 345–353. doi: 10.1016/j.tree.2010.01.007

Programa Nacional para el Control de la Abeja Africana-PNCAA. (2010). Situación actual y perspectiva de la apicultura en México. *Claridades Agropecuarias* 199, 3–34.

Quezada-Euán, J. J. G. (2007). A retrospective history of the expansion of Africanized honeybees in Mexico. *J. Apic. Res.* 46, 295–300. doi: 10.3896/ibra.1.46.4.16

Quezada-Euán, J. J. G. (2018). *Stingless bees of Mexico: The Biology, Management and Conservation of An Ancient Heritage*. Berlin: Springer.

Quezada-Euán, J. J. G., De Araujo-Freitas, C., Marrufo Olivares, J. C., Velásquez-Madrazo, A., and Medina-Medina, L. A. (2008). "Selección en colonias de abejas africanizadas: una alternativa viable para el trópico mexicano," in *Proceedings of the Memorias 2° Apimondia Simposio Mundial de Criadores de Abejas Reina e Inseminación Artificial*, Nayarit, 15–17.

Quezada-Euán, J. J. G., and Hinsull, S. M. (1995). Evidence of continued European morphometrics and mtDNA in feral colonies of honey bees (*Apis mellifera*) from the Yucatan peninsula. *Mexico. J. Apic. Res.* 34, 161–166. doi: 10.1080/00218839.1995.11100901

Quezada-Euán, J. J. G., May-Itzá, W., de, J., and González-Acereto, J. A. (2001). Meliponiculture in Mexico: problems and perspective for development. *Bee World* 82, 160–167. doi: 10.1080/0005772x.2001.11099523

Quezada-Euán, J. J. G., and May-Itza, W. J. (2001). Partial seasonal isolation of African and European-derived *Apis mellifera* (Hymenoptera: Apidae) drones at congregation areas from subtropical Mexico. *Ann. Entomol. Soc. Am.* 94, 540–544. doi: 10.1603/0013-8746(2001)094[0540:psioaa]2.0.co;2

Quezada-Euán, J. J. G., and Paxton, R. J. (1999). Rapid inter-generational changes in morphology and behaviour in colonies of Africanized and European honey bees (*Apis mellifera*) from tropical Yucatan. *Mexico. J. Apic. Res.* 38, 93–104. doi: 10.1080/00218839.1999.11101000

Quezada-Euán, J. J. G., Paxton, R. J., and Echazarreta, C. M. (1996). The distribution and range expansion of the Africanized honey bees (*Apis mellifera* L.) in the state of Yucatan. *Mexico. J. Apic. Res.* 35, 49–56.

Quezada-Euán, J. J. G., Pérez-Castro, E. E., May-Itzá, W., and de, J. (2003). Hybridization between European and African-derived honeybee populations (*Apis mellifera*) at different altitudes in Perú. *Apidologie* 34, 217–225. doi: 10.1051/apido:2003010

Ramírez-Flores, V. A., Villanueva-Gutiérrez, R., Roubik, C. W., Vergara, C. H., and Lara-Rodriguez, N. (2015). Topological structure of plant-bee networks in four Mexican environments. *Sociobiology* 62, 56–64.

Rangel, J., Giresi, M., Pinto, M. A., Baum, K. A., Rubink, W. L., Coulson, R. N., et al. (2016). Africanization of a feral honey bee (*Apis mellifera*) population in South Texas: does a decade make a difference? *Ecol. Evol.* 6, 2158–2169. doi: 10.1002/ece3.1974

Rinderer, T. E., and Collins, A. M. (1991). "Foraging behavior and honey production," in *The "African" Honey Bee*, eds M. Spivak, D. J. C. Fletcher, and M. D. Breed (Boulder, CO: Westview Press), 235–257. doi: 10.1201/9780429308741-12

Rinderer, T. E., Collins, A. M., Hellmich, R. L., and Danka, R. G. (1987). Differential drone production by Africanized and European honey bee colonies. *Apidologie* 18, 61–67. doi: 10.1051/apido:19870106

Rinderer, T. E., Collins, A. M., and Tucker, K. W. (1985a). Honey production and underlying nectar harvesting activities of Africanized and European honeybees. *J. Apic. Res.* 23, 161–167. doi: 10.1080/00218839.1985.11100666

Rinderer, T. E., and Hellmich, R. L. (1991). "The Process of Africanization," in *The "African" Honey Bee*, eds M. Spivak, D. J. C. Fletcher, and M. D. Breed (Boulder CO: Westview Press), 95–117. doi: 10.1525/ahu.2004.29.1.95

Rinderer, T. E., Hellmich, R. L., Danka, R. G., and Collins, A. M. (1985b). Male reproductive parasitism: a factor in the Africanization of European honey-bee populations. *Science* 228, 1119–1121. doi: 10.1126/science.228.4703.1119

Rinderer, T. E., Stelzer, J. A., Oldroyd, B. P., Buco, S. M., and Rubink, W. L. (1991). Hybridization between European and Africanized honey bees in the neotropical Yucatan peninsula. *Science* 253, 309–311. doi: 10.1126/science.253.5017.309

Rivera-Marchand, B., Giray, T., and Guzman-Novoa, E. (2008). The cost of defense in social insects: insights from the honey bee. *Entomol. Exp. Appl.* 129, 1–10. doi: 10.1111/j.1570-7458.2008.00747.x

Romero, M. J., and Quezada-Euán, J. J. G. (2013). Pollinators in biofuel agricultural systems: the diversity and performance of bees (Hymenoptera: Apoidea) on *Jatropha curcas* in Mexico. *Apidologie* 44, 419–429. doi: 10.1007/s13592-013-0193-x

Roubik, D. W. (1987). "Long-term consequences of the African honey bee invasion: implications for the United States," in *Proceedings of the Africanized Honey Bee Symposium*, (Park Ridge: American Farm Bureau), 46–54.

Roubik, D. W. (1989). *Ecology and Natural History of Tropical Bees*. Cambridge: Cambridge University Press.

Roubik, D. W. (2000). Pollination system stability in tropical America. *Conserv. Biol.* 14, 1235–1236. doi: 10.1046/j.1523-1739.2000.00016.x

Roubik, D. W. (2002). The value of bees to the coffee harvest. *Nature* 417, 708. doi: 10.1038/417708a

Roubik, D. W. (2009). Ecological impact on native bees by the invasive Africanized honey bee. *Acta Biol. Colomb.* 14, 115–124.

Roubik, D. W., and Villanueva-Gutiérrez, R. (2009). Invasive Africanized honey bee impact on native solitary bees: a pollen resource and trap nest analysis. *Biol. J. Linn. Soc.* 98, 152–160. doi: 10.1111/j.1095-8312.2009.01275.x

Roubik, D. W., and Villanueva-Gutiérrez, R. (2017). Have native Hymenoptera or Africanized bees become aggressive foragers due to resource competition? *Trends Entomol.* 13, 95–102.

Ruttner, F. (1988). *Biogeography and taxonomy of honey bees*. Berlin: Springer-Verlag.

Sader. (2020). *Servicio de información y estadística agroalimentaria (SIAP)*. Available online at: https://www.gob.mx/siap. (accessed September 1, 2020).

Santos, G. M. M., Aguiar, C. M. L., Genini, J., Martins, C. F., Zanella, F. C. V., and Mello, M. A. R. (2012). Invasive Africanized honeybees change the structure of native pollination networks in Brazil. *Biol. Invasions* 14, 2369–2378. doi: 10.1007/s10530-012-0235-8

Schneider, S. S., DeGrandi-Hoffman, G., and Smith, D. R. (2004). The African honey bee: factors contributing to a successful biological invasion. *Annu. Rev. Entomol.* 49, 351–376. doi: 10.1146/annurev.ento.49.061802.123359

Schneider, S. S., and McNally, L. C. (1992). Factors influencing seasonal absconding in colonies of the African honey bee, *Apis mellifera scutellata*. *Insect. Soc.* 39, 403–423. doi: 10.1007/bf01240624

Sheppard, W. S., Rinderer, T. E., Mazzoli, J. A., Stelzer, J. A., and Shimanuki, H. (1991a). Gene flow between African and European derived honey bee populations in Argentina. *Nature* 349, 782–784. doi: 10.1038/349782a0

Sheppard, W. S., Soares, A. E. E., De Jong, D., and Shimanuki, H. (1991b). Hybrid status of honey bee populations near the historic origin of Africanization in Brazil. *Apidologie* 22, 643–652. doi: 10.1051/apido:19910607

Silva-Contreras, A., Martínez-González, J., Cienfuegos-Rivas, E., López-Zavala, R., Tapia-González, J., and Parra-Bracamontes, G. (2019). Africanización de colonias en *Apis mellifera* L. (Hymenoptera: Apidae), presente en el ADN mitocondrial. *Abanico Veter.* 9, 1–11.

Smith, D. R., Taylor, O. R., and Brown, W. M. (1989). Neotropical Africanized honey bees have African mitochondrial DNA. *Nature* 339, 213–215. doi: 10.1038/339213a0

Spivak, M., Batra, S., Segreda, F., Castro, A. L., and Ramírez, W. (1989). Honey production by Africanized and European honey bees in Costa Rica. *Apidologie* 20, 207–220. doi: 10.1051/apido:19890303

Stort, A. C. (1975a). Genetic study of the aggressiveness of two subspecies of Apis mellifera in Brazil. II. Time at which the first sting reached the leather ball. *J. Apic. Res.* 14, 171–175. doi: 10.1080/00218839.1975.11099822

Stort, A. C. (1975b). Genetic study of the aggressiveness of two subspecies of Apis mellifera in Brazil. V. Number of stings in the leather ball. *J. Kans. Entomol. Soc.* 48, 381–387.

Tapia-González, J. M., Morfin, N., Macías-Macías, J. O., De la Mora, A., Tapia-Rivera, J. C., Ayala, R., et al. (2019). Evidence of presence and replication of honey bee viruses among wild bee pollinators in subtropical environments. *J. Invertebr. Pathol.* 168:107256. doi: 10.1016/j.jip.2019.107256

Tarpy, D. R., Caren, J. R., Delaney, D. A., Sammataro, D., Finley, J., Loper, G. M., et al. (2010). Mating frequencies of Africanized honey bees in the south western USA. *J. Apic. Res.* 49, 302–310. doi: 10.3896/ibra.1.49.4.02

Taylor, O. R. (1999). "Displacement of European honey bee subspecies by an invading African subspecies in the Americas," in *Apiculture for the 21st Century*, eds R. Hoopingarner and L. J. Connor (Cheshire, CT: Wicwas Press), 38–46.

Tehel, A., Brown, M. J. F., and Paxton, R. J. (2016). Impact of managed honey bee viruses on wild bees. *Curr. Opin. Virol.* 19, 16–22. doi: 10.1016/j.coviro.2016.06.006

Ueira-Vieira, C., Oliveira-Almeida, L., Correadealmeida, F., Marques, I., Brandemburgo, M. A. M., and Onetti, A. M. (2015). Scientific note on the first molecular detection of the acute bee paralysis virus in Brazilian stingless bees. *Apidologie* 46, 628–630. doi: 10.1007/s13592-015-0353-2

Unger, P., and Guzman-Novoa, E. (2012). Honey bee (Hymenoptera: Apidae) guarding behaviour may not be a rare task. *Can. Entomol.* 144, 538–541. doi: 10.4039/tce.2012.58

Uribe-Rubio, J. L., Guzman-Novoa, E., Hunt, G. J., Correa-Benítez, A., and Zozaya, J. A. (2003). The effect of Africanization on honey production, defensive behavior and size of honeybees (*Apis mellifera* L.) *in the Mexican high plateau. Veterinaria Mex.* 34, 47–59.

Uribe-Rubio, J. L., Guzman-Novoa, E., Vázquez-Peláez, C., and Hunt, G. J. (2008). Genotype, task specialization, and nest environment influence the stinging response thresholds of individual Africanized and European honeybees to electrical stimulation. *Behav. Genet.* 38, 93–100. doi: 10.1007/s10519-007-9177-9

Uribe-Rubio, J. L., Petukhova, T., and Guzman-Novoa, E. (2013). Genotype and task influence stinging response thresholds of honeybee (*Apis mellifera* L.) *workers of African and European descent*. *Open J. Ecol.* 3, 279–283. doi: 10.4236/oje.2013.34032

Vandame, R., and Palacio, M. A. (2010). Preserved honey bee health in Latin America: a fragile equilibrium due to low-intensity agriculture and beekeeping? *Apidologie* 41, 243–255. doi: 10.1051/apido/2010025

Vergara, C., Dietz, A., and De Leon, A. P. (1993). Female parasitism of European honey bees by Africanized honey bee swarms in Mexico. *J. Apic. Res.* 32, 34–40. doi: 10.1080/00218839.1993.11101285

Vergara, C. H., and Badano, E. I. (2009). Pollinator diversity increases fruit production in Mexican coffee plantations: the importance of rustic management systems. *Agric. Ecosyst. Environ.* 129, 117–123. doi: 10.1016/j.agee.2008.08.001

Whitfield, C. W., Behura, S. K., Berlocher, S. H., Clark, A. G., Johnston, J. S., Sheppard, W. S., et al. (2006). Thrice out of Africa: ancient and recent expansions of the honey bee. *Apis Mellif. Sci.* 314, 642–645. doi: 10.1126/science.1132772

Winston, M. L. (1979). Intra-colony demography and reproductive rate of the Africanized honeybee in South America. *Behav. Ecol. Sociobiol.* 4, 279–292. doi: 10.1007/bf00297648

Winston, M. L. (1992). *Killer bees: The Africanized Honey Bee in the Americas*. Massachusetts: Harvard University Press.

Winston, M. L., and Katz, S. J. (1981). Longevity of cross-fostered honey bee workers (*Apis mellifera*) of European and Africanized races. *Canad. J. Zool.* 59, 1571–1575. doi: 10.1139/z81-214

Woyke, J. (1984). Correlations and interactions between population, length of worker life and honey production by honeybees in a temperate region. *J. Apic. Res.* 23, 148–156. doi: 10.1080/00218839.1984.11100624

Zamora, O., Dominguez, R., Alanis-Gutierrez, L., and Quezada-Euán, J. J. G. (2008). Frequency of European and African-derived morphotypes and haplotypes in colonies of honey bees (*Apis mellifera*) from NW Mexico. *Apidologie* 39, 388–396. doi: 10.1051/apido:2008016

Zárate, O., De Araujo-Freitas, C., Medina, L. A., Velásquez, A., and Quezada-Euán, J. J. G. (2008). Phenotypic correlations of field and laboratory tests with honey production in Africanized honey bees (*Apis mellifera* L.). *Apidologie* 39, 523–530. doi: 10.1051/apido:2008036

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Island Invasions by Introduced Honey Bees: What Can Be Expected for Puerto Rico and the Caribbean?

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Apis species are a major component of pollinator faunas in their native and introduced habitats. A widespread concern is that non-native *Apis mellifera* may have negative effects on native pollinators and on plant reproduction. This is based on the assumptions that natural communities are at capacity, resource competition structures communities, native pollinators are more effective pollinators of native species, yet *A. mellifera* are superior competitors. The latter two assumptions are often true, but evidence from the Neotropics indicates that tropical communities are not tightly structured, and the foraging flexibilities of native bees maintain their populations. However, the less diverse and disharmonic biotas of islands may limit the buffering capacity of flexible behaviors. While few studies address these assumptions or the ecological and evolutionary consequences of *A. mellifera* to the flora and fauna of tropical islands, an accumulation of taxon-specific studies are suggesting that such effects run the spectrum from subtle and indirect to obvious and direct. A concerted research effort is needed to address the multitude of issues to develop strategies to ameliorate or enhance honey bee effects, or just let nature take its course.

Keywords: competition, loose niches, invasional meltdown, biological invasion, Island biology, *Apis mellifera*, Africanized honey bees, pollinator services

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INTRODUCTION

Native island biotas are depauperate compared to areas of similar size on continents, a phenomenon driven by immigration, extinction, isolation, area, habitat diversity, among other factors (MacArthur and Wilson, 1967; Whittaker et al., 2017). Island biotas also tend to be phylogenetically and functionally disharmonic since some taxa are more likely than others to arrive, survive and reproduce on an island (Taylor et al., 2019; König et al., 2020). This often leaves functional gaps on islands, such as predators, or certain taxa such as ants and palms (Weigelt et al., 2015). Given the lower diversity and disharmony of island biotas, we expect interaction networks to be loosely structured (Traveset et al., 2015).

Islands, especially in the tropics, are also disproportionately subject to human-assisted biological invasions (Rejmánek, 1996; Sax et al., 2002). Among the possible reasons for this are low biotic resistance, high resource availability, and high propagule pressure (e.g., Loope and Mueller-Dombois, 1989; Simberloff, 1995, 2009; Denslow, 2003). Consequently, local biotas have become a novel but functioning mix of native and introduced species (Lugo et al., 2018). Here I review the role of non-indigenous honey bees, *Apis mellifera* (HB) in these novel systems of tropical and subtropical islands, and what we can expect of HB interactions for the Caribbean and Puerto Rico in particular.

BEES AS INVADERS

Bees are among the most notable invasive insects. There are at least 80 species of bees recorded as non-indigenous invaders, many of them recorded in just the last 25 years (Russo, 2016). Bees are appreciated for their pollinator services in both natural and managed ecosystems, but there are costs. For all but two genera (*Apis* and *Bombus*), impacts are largely speculative as few studies have been published. However, the literature is replete with studies of invasive *Bombus* and *Apis*, and yet much more work needs to be done. Thus far, the negative impacts include competition with native species for floral resources and nest sites; displacement of native pollinators; transmission of pathogens and parasites; reduced reproductive success for native plants; disruption of plant-pollinator networks; hybridization with native species; and pollination services for invasive plants (Goulson, 2003; Thomson, 2004; Aizen et al., 2009; Abrol, 2012; Russo, 2016). The only negative impact that is unequivocally and consistently demonstrated is the transmission of pathogens and parasites to native bees (Paini, 2004; Russo, 2016).

Bees as Tropical and Subtropical Island Invaders

Nearly half the invasive bee species records are for islands, a third of these are exclusively island invaders, and most accidentally introduced. HB are one of the few notable exceptions (Russo, 2016). While islands may be susceptible to invasions because of "open niches," the relative frequency of floral visits by HB in island plant-pollinator networks generally do not attain the same level as they do in continental regions (Hung et al., 2018). Nevertheless, direct and indirect ecological impacts of non-indigenous bees through exploitative competition can be substantial.

Impacts of Invasive Honey Bees

What impacts do invasive HB have on the native flora and fauna? There is evidence that invasive HB can deplete pollen and nectar supplies available to native pollinators. If just one strong colony collects enough pollen in a single season to support 100,000 progeny of an "average" native bee, then this should have an impact (Cane and Tepedino, 2017). Competition for resources is suggested when resource niches overlap, visitation frequencies or resource use changes when HB arrive, and negative relationships between *Apis* abundance and native bees occur (Aizen and Feinsinger, 1994; Roubik and Villanueva-Gutiérrez, 2009; Thomson, 2016). HB pathogens can spread to native bees when resource use overlaps, a problem most evident with managed colonies (Fürst et al., 2014; Graystock et al., 2016; Grupe and Quandt, 2020). To understand HB impacts on native bee abundance and diversity, populations need to be monitored and experiments designed to test for resource competition as well as parasite/pathogen spillover with sufficient replication and duration. This is not often done (Paini, 2004).

The Neotropical invasion of Africanized Honey Bees (AHB) have displaced native bees from floral resources through their highly efficient foraging behavior (Moritz et al., 2005; Roubik, 2009), which could be demographically catastrophic for native bees (Minckley et al., 2003; Roulston and Goodell, 2011). Thus far, most continental studies of AHB show little or no negative demographic consequences for native solitary or social bees (Roubik, 1978, 1983, 1986, 2009; Roubik and Ackerman, 1987; Roubik and Wolda, 2001; Roubik and Villanueva-Gutiérrez, 2009). Native bees have the behavioral flexibility to shift to alternative food resources, which implies that floral resources are not limiting, and the bees have "loose niches" (Roubik, 1992), an idea supported by a global meta-analysis of pollination networks that revealed specialization decreases toward the tropics (Schleuning et al., 2012).

Impacts of Invasive Honey Bees on Tropical and Subtropical Islands

If islands with their disharmonic biotas have unfilled niches, then their native bee fauna may also have the capacity to shift to alternative resources when confronted by competition with non-indigenous species. On the other hand, the lower floral diversity on islands may leave fewer options when favored resources are usurped (Valido et al., 2019). Thus far, responses of native pollinators to invasions of generalist bees on tropical and subtropical islands are varied. Sometimes they appear to co-exist. Fiji has an endemic generalist bee, *Homalictus fijiensis* (Halictidae), which has been joined by three invasive generalist bees: HB, *Amegilla pulchra*, and *Braunsapis puangensis* (Apidae). All four species share resources and forage heavily from a broad range of non-native plants resulting in the endemic generalist being as connected as the three invasive species (Groutsch et al., 2019; Hayes et al., 2019). Such exploitation of alien plants by a native generalist is fairly typical of other islands (Olesen et al., 2002). In Hawai'i, *Metrosideros polymorpha*, the dominant native forest tree is visited at all elevations by both native and non-indigenous nectar feeders, with relative frequencies elevation-dependent. HB were most frequent at low and high elevations whereas the native bee, *Hylaeus* sp. (Colletidae) dominated at mid-elevations (Cortina et al., 2019). The drivers of these shifts are unknown.

Evidence for resource competition has been seen more clearly on other islands. Hansen et al. (2002) found that introduced HB on Mauritius competed with native birds for nectar resources with a likely net loss of seed production and possible demographic consequences to the birds. On Ogasawara Islands, Kato et al. (2018) found that outcomes are dependent on the degree of human disturbance. HB are the primary visitors to both native and non-native plants on islands with the most severe human impacts, whereas native bees dominate on islands with well-preserved floras. New Caledonia is particularly species-rich with high levels of endemism. In a survey of floral visitors to 95 native species, nearly half of the species were mellitophilous and these were visited predominantly by alien HB. As native bees do exist, the inference is that their populations have been reduced through competitive interactions (Kato and Kawakita, 2004). Thus, case histories show a broad spectrum of potential outcomes.

A surge in HB abundance through seasonal placement of apiaries can have strong negative impacts on biodiversity (Geslin et al., 2017). High-density beekeeping in natural areas of the Canary Islands has led to a drop in wild pollinator frequencies and fewer interaction links in pollination networks. Furthermore, reproductive success of native plants can decrease when heavily visited by HB, often a less effective pollinator (Valido et al., 2019). On islands where HB and non-native plants dominate, networks become more fragile. In the Seychelles and Hawai'i, networks with higher numbers of invasive plants have increased interspecific pollinations, and metrics such as connectivity, pollinator diversity and network redundancy are lower (Kaiser-Bunbury et al., 2011, 2017; Johnson and Ashman, 2019).

APIS MELLIFERA IN THE CARIBBEAN

The Caribbean is comprised of both oceanic and long-isolated continental islands. Surprisingly, our knowledge of HB interactions in the region is sparse. HB pathogens and parasites are present in the region (Rangel et al., 2018) but disease transmission dynamics and pathogen/parasite loads of wild bees are unknown. Hung et al. (2018) did a global meta-analysis of HB importance as pollinators in natural habitats and showed that the proportion of floral visits by HB was higher on continents than on islands. They had two examples from Caribbean islands: HB on both Jamaica and Dominica accounted for roughly 10% of the floral visits in the pollination networks studied (inferred from Hung et al., 2018, **Figure 1**, data from Ingversen, 2006). On the other hand, a comprehensive survey of bees and their host plants in the French West Indies revealed that HB visited 78% of the flora visited by bees (Meurgey, 2016). The consequences of HB activities in the Caribbean remain largely unknown, but studies from Puerto Rico indicate the dynamics that may exist.

Apis mellifera in Puerto Rico

The European honey bee (EHB) was established on Puerto Rico about 200 years ago (Mari Mut, 2018). In 1994, the Africanized honey bee (AHB) was unintentionally introduced and in a very short time fully supplanted EHB. Within 10 years AHB lost their aggressive defensive behavior resulting in bees that behave similarly to the original EHB populations (Cox, 1994; Rivera-Marchand et al., 2012). The change from EHB to AHB may have hidden consequences since AHB places greater emphasis on pollen foraging than EHB (Schneider et al., 2004); however, we have no means to determine whether a shift in pollinator services or any other interaction occurred since few studies of HB exist prior to the arrival of AHB. Nevertheless, we may expect that after 200 years, the flora and fauna of Puerto Rico have adjusted to the ubiquitous presence of HB (Moritz et al., 2005).

Effects on the Fauna of Puerto Rico

Snyder et al. (1987) noted an overlap in tree hole characteristics favored by EHB and the highly endangered Puerto Rican Parrot (*Amazona vittata*) and speculated "there can be little doubt that honey bees have been a significant factor in limiting the availability of good nest sites." While EHB may have had a role in the decline of the parrot, it likely pales in comparison to 150 years of deforestation, which left only 5% of original forest cover uncut by the 1940s (Wadsworth, 1950). Since then, forest cover has risen to 55%, making the outlook brighter for the parrot (Brandeis and Turner, 2013) and perhaps for feral AHB.

FIGURE 1 | Invasional meltdown in Puerto Rico. Africanized Honey Bees provides pollinator services for the bamboo orchid, *Arundina graminifolia*, thereby facilitating establishment and spread of the species across the island. Photo: James D. Ackerman.

Community assessments of plant-pollinator interactions in Puerto Rico where individual interactions can be extracted have not been published (e.g., Martín González et al., 2009, 2010). As in other islands worldwide (Crichton et al., 2018), Puerto Rico has at least one native super-generalist pollinator, *Xylocopa mordax* (Apidae), which uses at minimum 375 plant species for food and provides pollinator services for 43 fruit and seed crops (Jackson and Woodbury, 1976; Jackson, 1985). While there are ~42 bee species among islands of the Puerto Rican Bank (Genaro and Franz, 2008; Genaro, 2016; Gibbs, 2018), their floral resources are largely unknown. Without knowledge of plant-pollinator communities prior to HB introduction, we can only speculate what impact HB have had on native bees (Paini, 2004; Stout and Morales, 2009), and it may not have been negative as discovered in Fiji (Crichton et al., 2018).

Despite lacking knowledge of native bee populations in Puerto Rico before the arrival of either EHB or AHB, indirect evidence suggests HB may have had significant competitive effects. Fumero-Cabán (2019) conducted a 3-year study of the floral visitors to populations of a dry forest tree, *Guaiacum sanctum* (Zygophyllaceae) in Guánica, Puerto Rico, and nearby Mona Island, where HB do not occur. Frequencies of floral visitors were much higher in Guánica, but nearly all visits were by AHB (98%). Whereas, on Mona Island, *G. sanctum* had twice the diversity of flower visitors as seen in Guánica, and it took the 10 most frequent visitors to reach 98% of the total visits. If these data reflect pollinator diversity and abundance at each site, then *Apis* may have affected the native flower-visiting fauna of Puerto Rico's dry forests.

Effects on Pollinator Services in Puerto Rico

AHB are widespread across Puerto Rico, and because HB have been present for about two centuries, they should be fully integrated into local pollination networks. The following six case histories illustrate how AHB can have positive to neutral or even negative effects on the flora of the island, just as they have had on a global scale (Agüero et al., 2018).

HB have had a positive effect on the pollination of *Pitcairnia angustifolia* (Bromeliaceae), flowers of which fit the hummingbird pollination syndrome (Fumero-Cabán and Meléndez-Ackerman, 2007). As expected, the long-billed Green Mango (*Anthracothorax viridis,* Trochilidae) is a pollinator, but nectar-robbing Bananaquits (*Coereba flaveola,* Thraupidae) and pollen foraging HB also provide pollinator services despite their role differences. HB performed about 1/3 of the pollinations adding some redundancy which may buffer effects of catastrophic events.

In Guánica, AHB dominates the pollinator pool of the partially self-incompatible *G. sanctum*, but most visits were among flowers of the same tree, possibly resulting in reduced reproductive success due to inbreeding depression. However, AHB visitation frequencies were high, so that fruit and seed production were similar to populations where AHB did not occur (Fumero-Cabán, 2019).

The Sierra Palm (*Prestoea montana*) in Puerto Rico is a rainforest dominant and flowers abundantly from June to September. When the palms are in flower, numerous AHB forage over the inflorescences. Honey bees and small flies are presumed to be pollinators, but the frequency and effectiveness of floral visitors to this monoecious palm have yet to be quantified (Bannister, 1970). Fruit production is ∼3,800–5,000 per palm each year (Bannister, 1970; Gregory and Sabat, 1996) so pollinators are effective. If HB are the primary pollinators, then their role is critical for maintaining the structure and function of the rainforest.

Honey bees forage pollen from the hummingbird-pollinated cactus, *Melocactus intortus*. Flowers are self-compatible, but fruit set is lower when selfing occurs. Fagua and Ackerman (2011) found that AHB performed about 10% of the pollinations, yet when they calculated pollinator effectiveness taking into account seed viability, AHB did not affect reproductive success of the cactus. Here the role of AHB may be as inconsequential pollen thieves.

The role of HB as pollinators of the endangered tree, *Goetzea elegans* (Solanaceae), is equivocal. They often visit numerous flowers on a tree and are as effective at transferring pollen as the native pollinator, the Bannaquit, but flowers are self-incompatible and most AHB visits, unlike those of the bird, are within a tree (Caraballo-Ortiz and Santiago-Valentín, 2011; Caraballo-Ortiz et al., 2011). While AHB do perform some pollination services, many of them are incompatible pollinations, resulting in pollen wastage and perhaps stigma clogging, interfering with subsequent outcross pollinations.

Invasion of super-generalist pollinators such as HB has the potential to alter pollinator services and facilitate the invasion of alien plants by providing pollinator services that local pollinator would not otherwise perform (e.g., Morales and Aizen, 2006; **Figure 1**). The presence of HB in Puerto Rico has done this for several non-indigenous species, including the pigeon orchid (*Dendrobium crumenatum,* Orchidaceae). This epiphytic plant has escaped cultivation and has been spreading rapidly across moist and wet regions of the island. The nectar-rich flowers are pollinated by *Apis cerana and A. dorsata* in their native range (Brooks and Hewitt, 1909; Leong and Wee, 2013), but in the Caribbean, alien HB serve as a surrogate (Meurgey, 2016; Ackerman, 2017). While orchid enthusiasts may applaud orchid invasions, they serve as hosts to a native orchid-specialist weevil (*Stethobaris polita*, Curculionidae), whose populations have grown due to the increased food supply, resulting in reduced fruit set in native orchids (Recart et al., 2013).

WHAT WE NEED TO KNOW

HB presence in the Caribbean is likely permanent, but populations are expected to fluctuate. Aside from catastrophic losses and recovery from diseases, droughts, and hurricanes, resource availability should also change as forests mature from the height of deforestation, and non-indigenous species proliferate (Abelleira Martínez et al., 2015; Rojas-Sandoval and Acevedo-Rodríguez, 2015). HB studies have revealed loose niches on the species-rich continent, but has the lower diversity of islands been as forgiving? Will plant invasions, so severe on islands, exacerbate the problem or relieve it? Answers to these questions are uncertain since current evidence from tropical islands is sparse and mixed. The following lines of inquiry should be addressed: (1) *Basic bee diversity and dynamics*—What are the bee diversities among and within islands? How do abundances and population dynamics of native and feral HB vary across the landscape? What are the pathogen and parasite loads? When these are answered, then HB densities may be manipulated while local bee populations are monitored for responses. (2) *Community dynamics and resource availability*—What role does HB have in the structure, function, and stability of pollination networks across environmental conditions, vegetation types, forest-urban gradients, and agroecosystems? Do network metrics indicate a high potential for competitive interactions involving HB? Are niches loose enough to accommodate HB and native pollinators? (3) *Pollinator services*—Do HB have a detrimental or supplemental role in plant reproductive success, for native and non-indigenous species? Are native pollinators more or less efficient/effective pollinators in the island's agroecosystems than HB?

CONCLUSIONS

HB have been present in the Caribbean for at least 200 years and currently perform pollinator services for native plants, albeit imperfectly. Effects on native bee faunas are not clear, but the less diverse, disharmonic biotas of islands may limit the buffering capacity of loose niches against the competitive abilities HB. As the Caribbean has become a hotspot for biological invasions, the role of HB may become amplified resulting in further biological reorganization. This complicates the development of strategies to effectively foster local bee industries while minimizing negative consequences to natural and agro-ecosystems (e.g., Goulson and Hughes, 2015; Henry and Rodet, 2020). However, lessons learned from Caribbean studies will certainly inform dynamics seen in other tropical island systems.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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REFERENCES

Abelleira Martínez, O. J., Rodríguez, M. A., Rosario, I., Soto, N., López, A., and Lugo, A. E. (2015). Structure and species composition of novel forests dominated by an introduced species in northcentral Puerto Rico. *New For*. 39, 1–18. doi: 10.1007/s11056-009-9154-7

Abrol, D. P. (2012). "Consequences of introduced honeybees upon native bee communities," in *Pollination Biology: Biodiversity Conservation and Agricultural Production*, ed D. P. Abrol (London: Springer), 635–667.

Ackerman, J. D. (2017). Orchidées invasives: accélération de la colonization et de la propagation. *L'Orchidophile* 213, 167–173.

Agüero, J. I., Rollin, O., Torretta, J. P., Aizen, M. A., Requiier, F., and Garibaldi, L. A. (2018). Impactos de la abeja melífera sobre plantas y abejas silvestres en habitats naturales. *Ecosistemas* 27, 60–69. doi: 10.7818/ECOS.1365

Aizen, M. A., and Feinsinger, P. (1994). Habitat fragmentation, native insect pollinators, and feral honey bees in Argentine 'Chaco Serrano'. *Ecol. Appl*. 4, 378–392. doi: 10.2307/1941941

Aizen, M. A., Morales, C. L., and Morales, J. M. (2009). Invasive mutualists erode native pollination webs. *PLoS Biol*. 6:e31. doi: 10.1371/journal.pbio.0060031

Bannister, B. A. (1970). "Ecological life cycle of Euterpe globosa Gaertn.," in *A Tropical Rain Forest*, ed H. T. Odum (Washington, DC: Division of Technology Information; US Atomic Energy Commision), B299–B314.

Brandeis, T. J., and Turner, J. A. (2013). *Puerto Rico's Forests, 2009. Resource Bulletin, SRS-191.* Asheville, NC: US Department of Agriculture Forest Service, Southern Research Station.

Brooks, C. J., and Hewitt, J. (1909). Notes on the fertilization of a few orchids in Sarawak. *J. Straits Br. Roy. Asiatic Soc*. 54, 99–106.

Cane, J. H., and Tepedino, V. J. (2017). Gauging the effect of honey bee pollen collection on native bee communities. *Conserv. Lett*. 10, 205–210. doi: 10.1111/conl.12263

Caraballo-Ortiz, M. A., and Santiago-Valentín, E. (2011). The breeding system and effectiveness of introduced and native pollinators of the endangered tropical tree *Goetzea elegans* (Solanaceae). *J. Pollination Ecol*. 4, 26–33. doi: 10.26786/1920-7603(2011)5

Caraballo-Ortiz, M. A., Santiago-Valentín, E., and Carlo, T. A. (2011). Flower number and distance to neighbours affect the fecundity of *Goetzea elegans* (Solanaceae). *J. Trop. Ecol*. 27, 521–528. doi: 10.1017/S0266467411000289

Cortina, C. A., Aslan, C. E., and Litson, S. J. (2019). Importance of non-native honeybees (*Apis mellifera*) as flower visitors to the Hawaiian tree 'Ohi'a Lehua (*Metrosideros polymorpha)* across an elevation gradient. *Pacific Sci*. 73, 345–355. doi: 10.2984/73.3.3

Cox, B. (1994). AHB in Puerto Rico. *Am. Bee J*. 134:668.

Crichton, A., Francis, N., Doherty, S., Tuiwawa, M., Hays, S., Stevens, M. I., et al. (2018). Low endemic bee diversity and very wide host range in lowland Fiji: support for the pollinator super-generalist hypothesis in island biogeography. *Pac. Conserv. Biol*. 25, 135–142. doi: 10.1071/PC18037

Denslow, J. S. (2003). Weeds in paradise: thoughts on the invasibility of tropical islands. *Ann. Missouri Bot. Gard*. 90, 119–127. doi: 10.2307/3298531

Fagua, J. C., and Ackerman, J. D. (2011). Consequences of floral visits by invasive honeybee and ants to the hummingbird-pollinated, Caribbean cactus *Melocactus intortus*. *Plant Spec. Biol*. 26, 193–204. doi: 10.1111/j.1442-1984.2011.00319.x

Fumero-Cabán, J. J. (2019). *Reproductive ecology and population structure of Guaiacum sanctum (Zygophyllaceae) in two Caribbean islands: implications of conservation* (Dissertation). University of Puerto Rico, Río Piedras, San Juan, Puerto Rico.

Fumero-Cabán, J. J., and Meléndez-Ackerman, E. J. (2007). Relative pollination effectiveness of floral visitors of *Pitcairnia angustifolia* (Bromeliaceae). *Am. J. Bot*. 94, 419–424. doi: 10.3732/ajb.94.3.419

Fürst, M. A., McMahon, D. P., Osborne, J. L., Paxton, R. J., and Brown, M. J. F. (2014). Disease associations between honeybees and bumblebees as a threat to wild pollinators. *Nature* 506, 364–366. doi: 10.1038/nature12977

Genaro, J. A. (2016). Especies nuevas y nuevos registros de abejas para Las Antilles (Hymenoptera: Anthophila; Colletidae, Halictidae). *Novitates Caribaea* 10, 38–51. doi: 10.33800/nc.v0i10.28

Genaro, J. A., and Franz, N. M. (2008). The bees of Greater Puerto Rico (Hymenoptera: Apoidea: Anthophila). *Insecta Mundi* 569, digital commons.unl.edu/insectamundi/569

Geslin, B., Gauzens, B., Baude, M., Dajoz, I., Fontaine, C., Henry, M., et al. (2017). Massively introduced managed species and their consequences for pant-pollinator interactions. *Adv. Ecol. Res*. 57, 147–199. doi: 10.1016/bs.aecr.2016.10.007

Gibbs, J. (2018). Bees of the genus *Lasioglossum* (Hymenoptera: Halictidae) from Greater Puerto Rico, West Indies. *Eur. J. Taxon*. 400, 1–57. doi: 10.5852/ejt.2018.400

Goulson, D. (2003). Effects of introduced bees on native ecosystems. *Annu. Rev. Ecol. Evol. Syst*. 34. 1–26. doi: 10.1146/annurev.ecolsys.34.011802.132355

Goulson, D., and Hughes, W. O. H. (2015). Mitigating the anthropogenic spread of bee parasites to protect wild pollinators. *Biol. Conserv*. 191, 10–19. doi: 10.1016/j.biocon.2015.06.023

Graystock, P., Blane, E. J., McFrederick, Q. S., Goulson, D., and Hughes, W. O. (2016). Do managed bees drive parasite spread and emergence in wild bees? *Int. J. Parasitol. Parasit. Wildl*. 5, 64–75. doi: 10.1016/j.ijppaw.2015.10.001

Gregory, F. A., and Sabat, A. M. (1996). The effect of hurricane disturbance on the fecundity of Sierra Palms (*Prestoea montana*). *Bios* 67, 135–139.

Groutsch, J. K., Miller, N. C., Tuiwawa, M., Hayes, S., Stevens, M. I., and Schwarz, M. P. (2019). Not all exotic pollinator introductions are bad: an introduced buzz-pollinating bee *Amegilla pulchra* (Hymenoptera: Apidae) in Fiji indicates little potential for enhancing the spread of weeds. *Austral Entomol*. 58, 533–539. doi: 10.1111/aen.12346

Grupe, A. C., and Quandt, C. A. (2020). A growing pandemic: a review of *Nosema* parasites in globally distributed domesticated and native bees. *PLoS Pathog*. 16:e1008580 doi: 10.1371/journal.ppat.1008580

Hansen, D. M., Olesen, J. M., and Jones, C. G. (2002). Trees, birds and bees in Mauritius: exploitative competition between introduced honey bees and endemic nectarivorous birds? *J. Biogeogr*. 29, 721–734. doi: 10.1046/j.1365-2699.2002.00720.x

Hayes, S. E., Tuiwawa, M., Stevens, M. I., and Schwarz, M. P. (2019). A recipe for weed disaster in islands: a super-generalist native pollinator aided by a 'Parlourmaid' plant welcome new arrivals in Fiji. *Biol. Invasions* 21, 1643–1655. doi: 10.1007/s10530-019-01923-z

Henry, M., and Rodet, G. (2020). The apiary influence range: a new paradigm for managing the cohabitation of honey bees and wild bee communities. *Acta Oecol*. 105:103555. doi: 10.1016/j.actao.2020.103555

Hung, K.-U., Kinston, J. M., Albrecht, M., Holway, D. A., and Kohn, J. R. (2018). The worldwide importance of honey bees as pollinators in natural habitats. *P. Roy. Soc. Lond. B Bio*. 285:20172140. doi: 10.1098/rspb.2017.2140

Ingversen, T. T. (2006). *Plant-pollinator interactions on Jamaica and Dominica: the centrality, asymmetry and modularity of networks* (MSc thesis). University of Aarhus, Aarhus, Denmark.

Jackson, G. C. (1985). Additional host plants of the Carpenter Bee, *Xylocopa brasilianorum* (L.) (Hymenoptera: Apoida), in Puerto Rico. *J. Agri. U. Puerto Rico* 70, 255–265. doi: 10.46429/jaupr.v70i4.7102

Jackson, G. C., and Woodbury, R. O. (1976). Host plants of the carpenter bee, *Xylocopa brasilianorum* L. (Hymenoptera: Apoidea), in Puerto Rico. *J. Agr. U. Puerto Rico* 60, 639–660. doi: 10.46429/jaupr.v60i4.10512

Johnson, A. L., and Ashman, T.-L. (2019). Consequences of invasion for pollen transfer and pollination revealed in a tropical island ecosystem. *New Phytol.* 221, 142–154. doi: 10.1111/nph.15366

Kaiser-Bunbury, C. N., Mougal, J., Whittington, A. E., Valentin, T., Gabriel, R., Olesen, J. M., et al. (2017). Ecosystem restoration strengthens pollination network resilience and function. *Nature* 542, 223–227. doi: 10.1038/nature21071

Kaiser-Bunbury, C. N., Valentin, T., Mougal, J., Matatiken, D., and Ghazoul, J. (2011). The tolerance of island plant-pollinator networks to alien plants. *J. Ecol*. 99, 202–213. doi: 10.1111/j.1365-2745.2010.01732.x

Kato, M., and Kawakita, A. (2004). Plant-pollinator interactions in New Caledonia influenced by introduced honey bees. *Am. J. Bot*. 91, 1814–1827. doi: 10.3732/ajb.91.11.1814

Kato, M., Shibata, A., Yasui, T., and Nagamasu, H. (2018). Impact of introduced honeybees, *Apis mellifera*, upon native bee communities in the Bonin (Ogasawara) Islands. *Pop. Ecol*. 41, 217–228. doi: 10.1007/s101440050025

König, C., Weigelt, P., Taylor, A., Stein, A., Dawson, W., Essl, F., et al. (2020). Source pools and disharmony of the world's island floras. *Ecography*. 44, 44–55. doi: 10.1111/ecog.05174

Leong, T. M., and Wee, Y. C. (2013). Observations of pollination in the pigeon orchid, *Dendrobium crumenatum* Swartz (Orchidaceae) in Singapore. *Nat. Singapore* 6, 91–96. Available online at: https://lkcnhm.nus.edu.sg/wp-content/uploads/sites/10/app/uploads/2017/06/2013nis091-096.pdf

Loope, L. L., and Mueller-Dombois, D. (1989). "Characteristics of invaded islands, with special reference to Hawai'i," in *Biological Invasions: A Global Perspective*, eds J. A. Drake, F. DiCastri, R. H. Groves, F. J. Kruger, H. A. Mooney, M. Rejmánek, et al. (New York, NY: Wiley), 257–280.

Lugo, A. E., Winchell, K. M., and Carlo, T. A. (2018). "Novelty in ecosystems," in *The Encyclopedia of the Anthropocene, Vol. 3*, eds D. A. DellaSala and M. I. Goldstein (Oxford: Elsevier), 259–271.

MacArthur, R. H., and Wilson, E. O. (1967). *The Theory of Island Biogeography*. Princeton, NJ: University Press.

Mari Mut, J. A. (2018). *Abejas, Flores y Miel, 3rd Edn*. Aguadilla: Ediciones Digitales.

Martín González, A. M., Dalsgaard, B., and Olesen, J. M. (2010). Centrality measures and the importance of generalists in pollination networks. *Ecol. Complex*. 7, 36–43. doi: 10.1016/j.ecocom.2009.03.008

Martín González, A. M., Dalsgaard, B., Ollerton, J., Timmermann, A., Olesen, J. M., Andersen, L., et al. (2009). Effects of climate on pollination networks in the West Indies. *J. Trop. Ecol.* 25, 493–506. doi: 10.1017/S0266467409990034

Meurgey, F. (2016). Bee species and their associated flowers in the French West Indies (Guadeloupe, Les Saintes, La Désirade, Marie Galante, St Barthelemy and Martinique) (Hymenoptera: Anthophila: Apoidea). *Ann. Soc. Ent. France* 52, 209–232. doi: 10.1080/00379271.2016.1244490

Minckley, R. L., Cane, J. H., Kervin, L., and Yanega, D. (2003). Biological impediments to measures of competition among introduced honey bees and desert bees (Hymenoptera: Apiformes). *J. Kansas Entomol. Soc*. 76, 306–319. doi: 10.2307/25086117

Morales, C. L., and Aizen, M. A. (2006). Invasive mutualisms and the structure of plant-pollinator interactions in the temperate forests of north-west Patagonia Argentina. *J. Ecol*. 94, 171–180. doi: 10.1111/j.1365-2745.2005.01069.x

Moritz, R. F. A., Härtel, S., and Neumann, P. (2005). Global invasions of the western honeybee (*Apis mellifera*) and the consequences for biodiversity. *Ecoscience* 12, 289–301. doi: 10.2980/i1195-6860-12-3-289.1

Olesen, J. M., Eskildsen, L. I., and Venkatasamy, S. (2002). Invasion of pollination networks on oceanic islands: importance of invader complexes and endemic super generalists. *Divers. Distrib*. 8, 181–192. doi: 10.1046/j.1472-4642.2002.00148.x

Paini, D. R. (2004). Impact of the introduced honey bee (*Apis mellifera*) (Hymenoptera: Apidae) on native bees: a review. *Austral. Ecol*. 29, 399–407. doi: 10.1111/j.1442-9993.2004.01376.x

Rangel, J., González, A., Stoner, M., Hatter, A., and Traver, B. E. (2018). Genetic diversity and prevalence of *Varroa destructor*, *Nosema apis*, and *N. ceranae* in managed honey bee (*Apis mellifera*) colonies in the Caribbean island of Dominica, West Indies. *J. Apicult. Res.* 57, 541–550. doi: 10.1080/00218839.2018.1494892

Recart, W., Ackerman, J. D., and Cuevas, A. A. (2013). There goes the neighborhood: apparent competition between invasive and native orchids mediated by a specialist florivorous weevil. *Biol. Invas*. 15, 283–293. doi: 10.1007/s10530-012-0283-0

Rejmánek, M. (1996). "Species richness and resistance to invasion," in *Biodiversity and Ecosystem Processes in Tropical Forests*, eds G. Orians, R. Dirzo, and J. H. Cushman (New York, NY: Springer-Verlag), 153–172.

Rivera-Marchand, B., Oskay, D., and Giray, T. (2012). Gentle Africanized bees on an oceanic island. *Evol. Appl*. 5, 746–756. doi: 10.1111/j.1752-4571.2012.00252.x

Rojas-Sandoval, J., and Acevedo-Rodríguez. (2015). Naturalization and invasion of alien plants in Puerto Rico and the Virgin Islands. *Biol. Invas.* 17, 149–163. doi: 10.1007/s10530-014-0712-3

Roubik, D. W. (1978). Competitive interactions between neotropical pollinators and Africanized honey bees. *Science* 201, 1030–1032. doi: 10.1126/science.201.4360.1030

Roubik, D. W. (1983). Experimental community studies: time-series tests of competition between African and neotropical bees. *Ecology* 64, 971–978. doi: 10.2307/1937803

Roubik, D. W. (1986). Sporadic food competition with the African honey bee: projected impact on Neotropical social bees. *J. Trop. Ecol*. 2, 97–111. doi: 10.1017/S0266467400000699

Roubik, D. W. (1992). "Loose niches in tropical communities: why are there so may trees and so few bees?," in *Resource Distribution and Animal-Plant Interactions*, eds M. D. Hunter, T. Ohgushi, and P. W. Price (New York, NY: Academic Press), 327–354.

Roubik, D. W. (2009). Ecological impact on native bees by the invasive Africanized honey bee. *Acta Biol. Colombiano* 14, 115–124.

Roubik, D. W., and Ackerman, J. D. (1987). Long-term ecology of euglossine orchid-bees (Apidae: Euglossini) in Panama. *Oecologia* 73, 321–333. doi: 10.1007/BF00385247

Roubik, D. W., and Villanueva-Gutiérrez, R. (2009). Invasive Africanized honey bee impact on native solitary bees: a pollen resource and trap next analysis. *Biol. J. Linn. Soc*. 98, 152–160. doi: 10.1111/j.1095-8312.2009.01275.x

Roubik, D. W., and Wolda, H. (2001). Do competing honey bees matter? Dynamics and abundance of native bees before and after honey bee invasion. *Popul. Ecol.* 43, 53–62. doi: 10.1007/PL00012016

Roulston, T. H., and Goodell, K. (2011). The role of resources and risks in regulating wild bee populations. *Annu. Rev. Entomol*. 56, 293–312. doi: 10.1146/annurev-ento-120709-144802

Russo, L. (2016). Positive and negative impacts of non-native bee species around the world. *Insects* 7:69. doi: 10.3390/insects7040069

Sax, D. F., Gaines, S. D., and Brown, J. H. (2002). Species invasions exceed extinctions on islands worldwide: a comparative study of plants and birds. *Amer. Nat*. 160, 766–783. doi: 10.1086/343877

Schleuning, M., Fründ, J., Kelin, A.-M., Abrahamczyk, S., Alarcón, R., Albrecht, M., et al. (2012). Specialization of mutualistic interaction networks decreases toward tropical latitudes. *Curr. Biol.* 22, 1925–1931. doi: 10.1016/j.cub.2012.08.015

Schneider, S. S., DeGrandi-Hoffman, G., and Smith, D. R. (2004). The African Honey Bee: factors contributing to a successful biological invasion. *Annu. Rev. Entomol.* 49, 351–376. doi: 10.1146/annurev.ento.49.061802.123359

Simberloff, D. (1995). Why do introduced species appear to devastate islands more than mainland areas? *Pac. Sci.* 49, 87–97.

Simberloff, D. (2009). The role of propagule pressure in biological invasions. *Annu. Rev. Ecol. Evol. Syst.* 40, 81–102. doi: 10.1146/annurev.ecolsys.110308.120304

Snyder, N. E. R., Wiley, J. W., and Kepler, C. B. (1987). *The Parrots of Luquillo: Natural History and Conservation of the Puerto Rican Parrot*. Los Angeles, CA: Western Foundation of Vertebrate Zoology.

Stout, J. C., and Morales, C. L. (2009). Ecological impacts of invasive alien species on bees. *Apidologia* 40, 388–409. doi: 10.1051/apido/2009023

Taylor, A., Weigelt, P., König, C., Zotz, G., and Kreft, H. (2019). Island disharmony revisited using orchids as a model group. *New Phytol.* 223, 597–606. doi: 10.1111/nph.15776

Thomson, D. M. (2004). Competitive interactions between the invasive European honey bee and native bumble bees. *Ecology* 85, 458–470. doi: 10.1890/02-0626

Thomson, D. M. (2016). Local bumble bee decline linked to recovery of honey bees, drought effects on floral resources. *Ecol. Lett.* 19, 1247–1255. doi: 10.1111/ele.12659

Traveset, A., Tur, C., Trøjelgaard, K., Heleno, R., Castro-Urgal, R., and Olesen, J. M. (2015). Global patterns of mainland and insular pollination networks. *Global Ecol. Biogeogr.* 25, 880–890. doi: 10.1111/geb.12362

Valido, A., Rodríguez-Rodríguez, M. C., and Jordano, P. (2019). Honeybees disrupt the structure and functionality of plant-pollinator networks. *Sci. Rep.* 9:4711. doi: 10.1038/s41598-019-41271-5

Wadsworth, F. H. (1950). Notes on the climax forest of Puerto Rico and their destruction and conservation prior to 1900. *Caribb. Forester* 11, 47–48.

Weigelt, P., Kissling, W. D., Kisel, Y., Fritz, S. A., Karger, D. N., Kessler, M., et al. (2015). Global patterns and drivers of phylogenetic structure in island floras. *Sci. Rep.* 5:12213. doi: 10.1038/srep12213

Whittaker, R. J., Fernández-Palacios, J. M., Matthews, T. J., Borregaard, M. K., and Triantis, K. A. (2017). Island biogeography: taking the long view of nature's laboratories. *Science* 357:eaam8326. doi: 10.1126/science.aam8326

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Community and Species-Level Changes of Insect Species Visiting *Mangifera indica* Flowers Following Hurricane María: "The Devil Is in the Details"

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Mangifera indica is a widespread economically important tropical fruit. An ongoing study at the Juana Diaz Experimental Station in Puerto Rico aims to understand the factors that influence local pollination success and fruit yields in four fields each hosting a different mango cultivar (Keitt, Kent, Tommy Atkins, and Julie) at different temporal scales. Here we describe the results of insect collection campaigns that evaluated flower visitor communities of these fields (from January to April) in the seasons of 2017 (before Hurricane Maria), 2018 (after Hurricane Maria) and 2019 (2 years after Hurricane Maria). We expected a reduction in diversity, abundance and yields and even changes in composition following the hurricane events of 2017. Over the 3 years, plants were visited by a combined total of 50 insect species, mostly Diptera (also the most abundant), Hymenoptera, Coleoptera, and Lepidoptera. The relative abundances of insect communities changed but overall species richness of insect communities appeared to be recovering by 2019. A clear decline in overall crop yields for two of the four fields (hosting Kent and Tommy Atkins) was seen in 2018 but then recovered in one and surpassed pre-hurricane levels in another in 2019. Mango trees experienced an increase in the abundance for all insect groups in 2019 following the 2018 decline and only one field (hosting Kent) experienced significant species richness declines in 2018. Two of the most dominant insects, *Palpada vinetorum* (Diptera) and *Apis mellifera* (Hymenoptera), showed a "reduction-recovery" pattern for the period of 2018–2019 but not so for *Cochliomyia minina* which was very abundant in 2018 in three out of four cultivars but then returned to pre-hurricane levels in 2019. In 2017, the trees exposed to higher richness and abundance of species experienced higher yields regardless of cultivars but these relationships when present were often weaker in 2018 (post-hurricane) and 2019 and not all cultivars were equally successful at attracting the same levels of diversity and abundance of insects. Our results do support the importance of pollinator diversity and abundance to improve agricultural yields. They also emphasize that within the context of future extreme atmospheric events, that there needs to be an understanding of not only how these pollinator communities may recover from these events but also of how individual pollinators (vs. other factors) may influence plant yields to develop informed management strategies following such events.

Keywords: Caribbean, pollinator, disturbance, insect diversity, resilience, Diptera, Apis

INTRODUCTION

Observed declining trends in the diversity and abundance of pollinators, may threaten global economies and our capacity to meet increasing global food demands (Potts et al., 2010a,b; Cameron et al., 2011; Bartomeus et al., 2013; Jevanandam et al., 2013; Aizen et al., 2019). A significant portion of the world's crop production (35%) depends on animal pollination (Klein and Bernard, 2007) and many of those are dependent on insect pollinator (Rader et al., 2013). In the United States alone, the value of this "pollination service" to the agriculture has been estimated at $43B (Losey and Vaughan, 2006). While managed pollinators like honey bees are main contributors of worldwide agricultural production (Potts et al., 2010b), studies suggest that wild pollinators are also important as they can increase agricultural yield and quality of fruit crops (e.g., Garibaldi et al., 2013; Garratt et al., 2014; Rader et al., 2016). Given the importance that pollination services represent to food and economic security, calls for strategies to conserve, manage and monitor pollinator abundance and biodiversity have been on the rise to reduce potential risks (Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services [IPBES], 2016). A core element of the International Pollinator Initiative's 2013–2018 action plan led by the Food Agriculture Organization (FAO) and the Convention for Biological Diversity (CBD) centers around activities related to monitoring research and assessment on the status and trends of pollinators and pollination (Convention on Biological Diversity, 2018). Information on the diversity and abundance of pollinator species is an important step toward understanding the stability and resilience of crop pollination services (Rader et al., 2013; Rogers et al., 2013). Developing monitoring strategies will be critical when evaluating pollinator management strategies especially in the face of climate variability and the prospect of increases in extreme atmospheric events world-wide.

Predicting the effects of extreme atmospheric events such as hurricanes on the abundance and diversity of insect pollinators is complex. Indeed the literature offers no agreement about the effects of hurricanes on insect communities with some species increasing while others decreased in abundances following these events (Torres, 1992; McGinley and Willig, 1999; Schowalter and Ganio, 1999; Koptur et al., 2002; Gandhi et al., 2007; Spiller et al., 2016). Some responses are modulated by spatial differences in microhabitat (e.g., Schowalter et al., 2017) and others by species interactions within and between trophic levels (e.g., Schowalter, 2012; Novais et al., 2018). From the perspective of diversity changes, it has been argued that while extreme weather events (hurricanes and frosts) often result in immediate reductions of alpha diversity, insect assemblages tend to be fairly resilient returning to pre-disturbance levels within 1–5 years (Marquis et al., 2019; Chen et al., 2020). It should be noted that most studies evaluating the effects of these events on insect communities do not often include pollinators.

Studies addressing the dynamics of insect pollinators following hurricane events have a narrow geographic scope and most are focused on non-agricultural systems. One study explicitly evaluated changes in insect pollinator assemblages in Florida (United States) after hurricanes Frances and Jeanne and showed a decline in species diversity accompanied by a reduction in the reproductive success of *Laguncularia racemosa* (Landry, 2013). In a second study, the insect pollinator community visiting *Ardisia escallonioides* following Hurricane Andrew in 1998, changed little in composition and abundance of their generalist pollinators (Pascarella, 1998). A third study, also in Florida, showed that after hurricane Andrew populations of *Pegoscapus jimenizeii* (fig wasps), a specialized pollinator of *Ficus aurea,* dropped but were able to recover in only 5 months (Bronstein and Hossaert-McKey, 1995). On the other hand, hurricanes across Yucatan, México, are one of several factors contributing to the decline of feral and managed colonies of stingless bees with generalist species persisting even when some of their food species disappear (Villanueva-Gutierrez et al., 2005; Roubik and Villanueva-Gutierrez, 2009). Clearly more studies are needed to evaluate the potential role of these events on pollinator communities and how these may affect plant reproduction. This would be particularly important for agricultural systems when their production is dependent on animal pollination.

Mangifera indica (mango), is native to Asia but has been widely dispersed in tropical and subtropical areas around the world (Jiron and Hedström, 1985; Galán, 2009). It is an economically important crop worldwide with over 1,000 cultivars (Galán, 2009). The Asian continent is the principal producer (75%) for this crop plant followed by Latin America and the Caribbean region (14%) and Africa (10%) (FAOSTAT, 2000). The scientific literature suggests that flower visitors of *M. indica* are quite diverse taxonomically and that there is considerable variation among geographic regions. For example, studies in Taiwan and Australia reported that Hymenoptera were the only flower visitors and potential pollinators of *M. indica* (Anderson et al., 1982; Hsin Sung et al., 2006) whereas in India, Diptera were named as dominant flower visitors (Singh, 1988; Ramírez and Davenport, 2016). Meanwhile, studies in Israel and Africa, showed that the main visitors of mango plants were a mixture of both Hymenoptera and Diptera species (Dag and Gazit, 2000) or Hymenoptera, Diptera, and Coleoptera (Carvalheiro et al., 2010, 2012) while in some areas, *Apis* bees were important pollinators (Wongsiri and Chen, 1995). The combined data suggest that *M. indica* flowers can attract a large diversity of floral visitors and therefore has a pollination system that may be generalized (Anderson et al., 1982; De Siqueira et al., 2008; Fajardo et al., 2008; Corredor and García, 2011; Kumar et al., 2012). While generalized pollination systems may offer resilience capacity within the context of environmental change (Waser et al., 1996; González-Varo et al., 2013), this may not always be the case (Memmott et al., 2007) or may not occur at a speed that is necessary in economically important species.

In the Caribbean region, the island of Puerto Rico is among the principal producers of *M. indica* (Central America Data, 2016) and Puerto Rico's climate is changing. Since 1950, air temperatures have increased by 2°C as well as the frequency and intensity of extreme climatic events (e.g., heat waves, droughts, and tropical storms) (Gould et al., 2015). Indeed, tropical storms and hurricanes have become more common and more intense during the past two decades (Environmental Protection Agency [EPA], 2016) and this trend is expected to continue according to most climate models (see reviews by Gould et al., 2015; Fain et al., 2018). Following hurricane events, flower resources available to pollinators may be reduced through the direct impact to plants (e.g., via mortality or defoliation; Rathcke, 2000) for pollinating insects. Hurricane-driven ecosystem modification may also reduce the availability of suitable habitat (e.g., flooding of ground nesting sites for insects, Savage et al., 2018) and result in declines in pollinating fauna (Landry, 2013). Therefore, both mango trees and their pollinator communities can be affected by the current trends in atmospheric events and these could affect local yields in Puerto Rico. Detecting declines in pollinator abundances and reproductive success of *M. indica* as a result of these events has yet to be documented in hurricane prone areas. On the other hand, predictions about the effects of hurricanes effects on plant-pollinator interactions are not necessarily straightforward as the impacts on pollinator abundances by these phenomena have not been documented frequently and only for a limited number of taxa.

In 2017, hurricanes Irma (Category 1 on land) and Maria (Category 4 on land) hit the island of Puerto Rico leading to considerable changes in the island's green landscape and changes in ambient temperature. It was estimated that when Hurricane Maria hit Puerto Rico on 20 September 2017, 23–31 million trees island-wide were severely damaged or killed (Feng et al., 2018) with some areas in Puerto Rico reporting ambient temperature increases of up to 4°C for a full year (U.S. Forest Service, 2018). Such stresses could reduce the ability of plants to produce fruits through a reduction of photosynthetic resources available to produce flowers and fruits and through a reduction of their pollinator communities (Rathcke, 2000). Following the 2017 hurricane season, we took advantage of an ongoing monitoring study of the flower visitors of four cultivars of *M. indica* on the island of Puerto Rico to address the potential influence of these hurricane events on the flower visitor communities of *M. indica* cultivars and how hurricane-influenced changes in these communities related to plant reproductive success. We specifically asked if insect communities visiting mango flowers varied significantly across years and how these changes related to plant reproductive success. One hypothesis is that when they occur, hurricanes could lead to a reduced diversity and abundance of local *M. indica* pollinators and result in reduced yields. At the same time, a current paradigm is that pollinator diversity and not just abundance may help increase pollinator services and plant reproductive success (Gómez et al., 2007; Albrecht et al., 2012). Thus, a minor goal was to evaluate the functional relationships between reproduction and pollinator diversity and abundance. We also sought to determine how these relationships may change following hurricane events and whether they differed among four fields each of which hosted a different mango cultivar. Lastly, we examined the relative importance of *Apis mellifera* to mango reproduction. *Apis mellifera* is often seen as a replacement for local species but managed *A. mellifera* failed to improve yields for *M. indica* in Africa (Carvalheiro et al., 2010). Furthermore, we know little on how *A. mellifera* responds to climatic events in the Caribbean Region. Results of this study provide insights into the dynamics of insect communities visiting flowers of *M. indicia* within the context of extreme weather events (i.e., hurricanes). From a management perspective, our results may help inform proposed strategies for managed pollination following such events.

MATERIALS AND METHODS

Study Site

We conducted our study at the Juana Díaz Agricultural Experiment Station (JDAES) in the island of Puerto Rico (18° 01′N, 66° 31′W) (**Figure 1**). The Station covers 111.23 ha and was established in 1950 by the University of Puerto Rico, Mayagüez Campus. It is located in the Southern side of the island which lies within a subtropical dry forest life zone (Ewel and Whitmore, 1973). The site has an average monthly temperature that ranges from 22 to 33°C and an annual rainfall of 977.1 mm (Harmsen et al., 2014). According to the USDA Survey, JDAES soils are classified as "Prime" with irrigation systems that are primarily driven by extracting groundwater (Beinroth et al., 2003). Since 1968, the Station has maintained a mango germplasm which today has 84 cultivars that occupy approximately 14.16 ha (**Figure 1**). Data was collected in four fields within the germplasm area and each field hosted a different cultivar (see below). For simplicity, each field is named after the cultivar it hosted.

Study System

Mangifera indica L. (Anacardiaceae) is a perennial tree that produces highly branched inflorescences (Coetzer et al., 1995; Goguey, 1997) with flowers that range from 5 to 10 mm in diameter that carry five green sepals and five petals with yellow nectary discs and colors ranging from white to yellow, pink or red (Galán, 2009; **Figure 1**). Inflorescences carry both hermaphroditic and male flowers. In hermaphroditic flowers the ovary is globose with a single stigma (Ramirez and Davenport, 2010). The Keitt cultivar evaluated in this study is derived from the Mulgoba cultivar, which has a moderate to tall tree height (9.1–40 m), an open canopy, and fruit sizes ranging from 13 to 15 cm (Campbell, 1992). This is the most planted cultivar on the southside of the island. The Kent cultivar evaluated here was derived from the Brooks cultivar and can reach heights of 40 m,

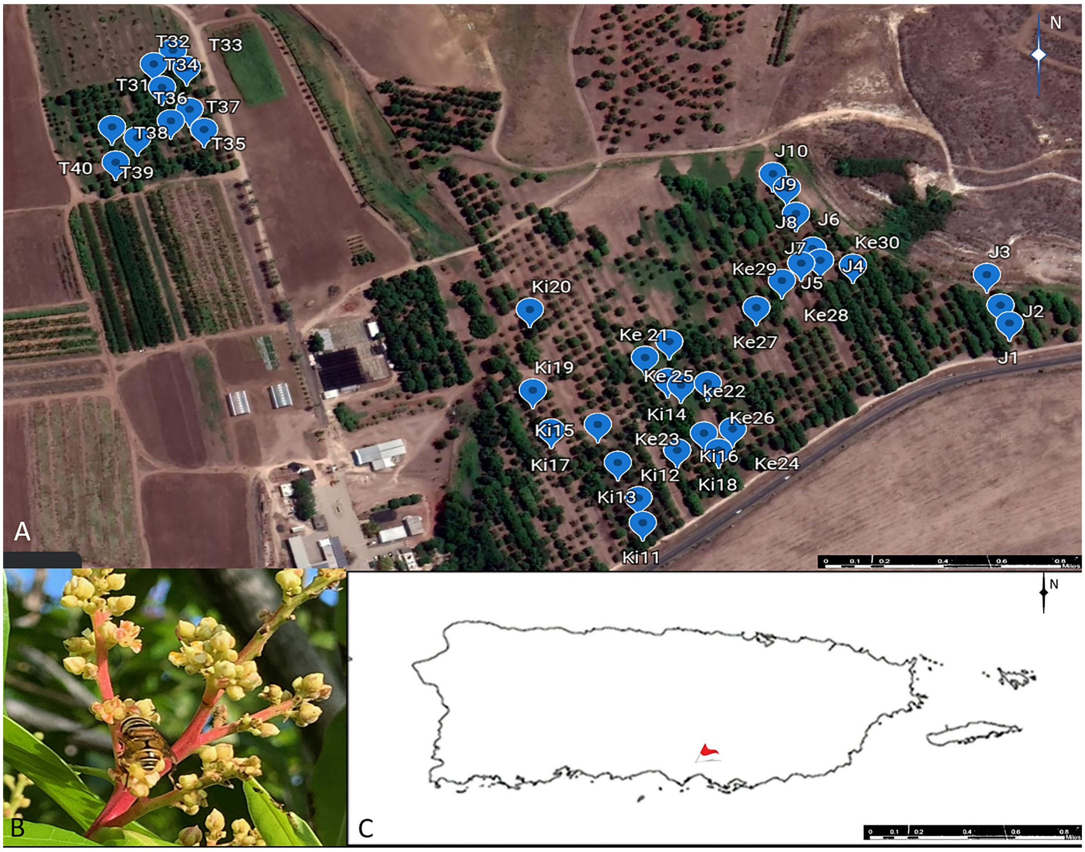

FIGURE 1 | (A) Layout of the Juana Díaz Agricultural Experimental Station indicating the location of the mango germplasm and the four fields [Julie (J), Keitt (Ki), Kent (Ke), Tommy Atkins (T)] used in this study. **(B)** *Palpada vinetorum* visiting inflorescences of the Julie fields. **(C)** Location of the Agricultural Experiment Station Juana Díaz, PR. Basemap was obtained from Google Earth Pro, (November 27, 2020) Juana Diaz, Puerto Rico. 18°01'37 73"N, 66° 31' 23 56" W Maxar Technologies, https://earth.google.com (February 2, 2021).

has a foliage that looks more compact, and produces fruit sizes ranging from 11 to 13 cm that are a favorite in Latin America (Campbell, 1992). The Tommy Atkins cultivar was derived from the Haden cultivar and produces a dense, rounded top tree with a fruit that can measure between 12 and 14.5 cm and is often preferred for its fruit color (skin orange-yellow, crimson, or dark red blush color) for export (Campbell, 1992). Julie is the smallest of the four cultivars studied. It grows only up to 3.3 m tall and produces smaller fruits relative to the remaining three cultivars. This is the main mango exported from the West Indies to Europe, believed to have been imported from Jamaica to other countries in the Caribbean and a favorite throughout the Caribbean region (Morton, 1987). For any of these cultivars, flowering has historically occurred within the course of 5–6 weeks during the dry months, which in Puerto Rico occurs between the months of January and April although some variation of the onset of flowering may occur depending on the year.

Data Collection

We collected insects visiting the flowers of 10 plants of each of the four *M. indica* fields (*N* = 40 plants) three days a week during their annual flowering cycles. In the 2017, and 2018 seasons, flowering occurred from January to April whereas for the 2019 season, flowering started early and ran from the last week of December and continued until April. Trees in the general mango germplasm were planted in rows with a 7 m minimum distance from one planted tree to another and a total area surveyed per cultivar of 0.70 ha. Likewise, cultivars varied in their distance from each other: 64.7 m between the Keitt and Kent cultivars, 477.0 m between Keitt and Tommy Atkins and 150.4 m between Kent and Julie (**Figure 1**). For each tree in a cultivar, we selected five inflorescences to be the focus of the insect collections (50 inflorescences/cultivar). Inflorescences on a tree were observed for insect visitors that were seen performing legitimate visitations (i.e., when the corolla was open and the visitor was collecting resources (nectar or pollen from it). Once detected, insects were collected by sweeping an entomological net over the inflorescence. Observations and collections were done three days every week: Monday, Wednesday and Friday. Each day, collection was carried out between 09:00 h and 14:00 h following a systematic scheme. The day was divided into 1-h periods with four 15-min intervals each assigned to a given cultivar. Within each 15-min interval, inflorescences for all trees were observed (1.5 min/tree) and insects visiting flowers in legitimate ways were collected in separate bags recording the time and the tree ID on each bag. At each hour period, the order of cultivars was rotated, and the order of trees within a cultivar as well to make sure that cultivars and their respective trees were observed at different daytime periods within the flowering season. All collected insects were taken to the Laboratory to be mounted or preserved in 75% alcohol. All specimens were identified to species except for one which was identified to genus. For each observed tree we also recorded the number of inflorescences per tree during the flowering peak and collected and weighed fruits to generate a value for the number of fruits per tree and fruit yield (kg).

Data Analyses

For each field (Julie, Keitt, Kent, Tommy Atkins), an insect data matrix was constructed with the variables year, field, collection week (1, 2, 3, etc.), pooled insect abundance for each *M. indica* field. This abundance matrix was then analyzed with non-metric multidimensional scaling (NMDS) analysis and time vector overlays (using year as the time variable) (Clarke, 1993; McCune and Grace, 2002) to evaluate potential differences in flower visitor communities among fields and across time periods. This analysis was carried out in PC-ORD version 5.0 (McCune and Mefford, 2006) using the Bray–Curtis similarity index and performing 250 iterations with randomized data to select a dimensionality, and then performing one iteration with the chosen dimensionality to find a stable solution with minimal stress (McCune and Grace, 2002). To explore which species best explained the variance among insect communities in multivariate space, we used Kendall correlation analyses that specifically tested associations between species abundance and the first and second NMDS axes (McCune and Grace, 2002). For these analyses we considered species abundances with a Kendall correlation coefficients of $(r) \geq 0.4$ to be significantly correlated with the NMDS multivariate axes and later used the three most dominant ones (and the most consistent in activity across cultivars) to evaluate their association with plant reproductive success (see below). For the purpose of this work, we provide results on total insect species diversity (hereafter richness) and abundance as well as abundances for the three most dominant species as these were the most consistent visitors for all four fields.

Our intent was to evaluate differences in total insect abundance, species richness as well as the abundance of dominant species between years and fields and how these variables related to mango reproduction. We first tested for the presence of systematic spatial variation of variables which could influence statistical results when using ordinary least square methods (Dormann et al., 2007). To that effect, we did exploratory analyses of Moran's I values for all numeric variables in GeoDa (Anselin et al., 2006) first pooling data from all cultivars to check for spatial autocorrelation issues at a landscape level. We then used values for each individual cultivar to test for autocorrelation at a local scale. Moran's I analyses did reflect spatial autocorrelation issues at the landscape scale but not at local scale (i.e., within a cultivar). Therefore, first we performed "permutational multivariate analysis of variance" (PERMAVOVA) using the Bray-Curtis dissimilarity index to test for multivariate dissimilarities integrating variables related to pollinator species richness and abundance (insect species richness, abundance, and individual abundances of dominant insect species) with year, field and their interaction as main effects. Unlike a regular MANOVA, PERMANOVA is not dependent on the correlation structure among groups (Anderson, 2014). To visualize relationships based on dissimilarity, we generated a dendrogram from a cluster analysis based on the unweighted pair group method with arithmetic mean (UPGMA) and the Bray Curtis dissimilarity index using MSVP v3.22 (Kovach, 2007). We followed PERMANOVA with a series of complementary analyses to evaluate changes in insect community variables and plant reproduction and the relationship among these at the field scale to circumvent spatial autocorrelation issues and using ln transformed variable values to meet the requirement of parametric tests. To evaluate the influence of insect abundance and diversity and the number of inflorescences per tree on the number of fruits/tree and total yield (kg/tree) of each mango field each year, we used generalized linear models to evaluate how the data fitted the models under different distributions (poisson, normal, exponential and negative binomial) and using the lowest AICc value (an adjusted AIC to account for small sample sizes, Cavanaugh, 1997) as our selection criteria for the best model and using a 2 unit minimum criterion for model selection (Burnham and Anderson, 2002). For models under a normal distribution (all but two), ordinary least square models were also run to generate R^2 estimates. For each field, we ran repeated measures ANOVAs to test for univariate differences across years in total insect species richness and abundance, fruit yield and the number of inflorescences as well as pairwise Pearson correlation analyses to test for associations between fruit yield with the number of fruits and also between the number inflorescences with fruit yield. We also ran multiple correlation analyses to test for associations between the abundance of each of the three dominant insect species observed for each tree and fruits yield per tree for each field and each year. To account for a potential increase in type II errors from multiple analyses ($N = 28$ correlations), p-values were corrected using Bonferroni corrections (Hammer et al., 2001). GLM Analyses were run in JMP v. 13.1 (SAS Cary Institute Inc, 2019) while PERMANOVA, Repeated measures ANOVAs and correlations were run in in InfoStat (Di Rienzo et al., 2018).

RESULTS

Insect Community Composition

A grand total of 3,795 individuals distributed across 50 insect species were collected in four mango cultivars (distributed across four fields) across the 3 years of the study (2017: $N = 920$; 2018: $N = 1,318$; 2019: $N = 1,557$). NMS analyses showed that 94.3% of the temporal variation in the insect community composition was explained by a two-dimensional solution with most of the variation (70.4%) explained by Axis 1 (**Figure 2**). The NMS analysis showed that observations for different the different fields (Julie, Keitt, Kent and Tommy) based on species similarity tended to form clusters around time periods and not around the fields themselves. That is, within a given year

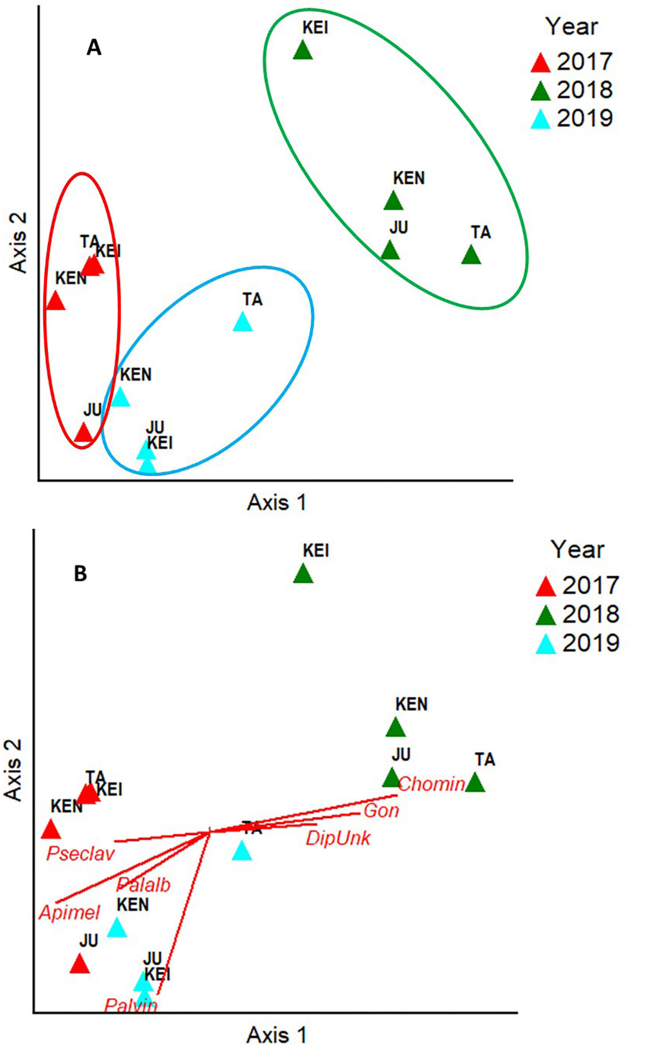

FIGURE 2 | Non-parametric multidimensional scaling analysis (NMS) based on Bray-Curtis similarity index evaluating differences in insect species composition among years and fields (Julie, Keitt, Kent, Tommy Atkins). **(A)** Observation groupings based on within year similarities. **(B)** Correlations lines showing the strongest associations between insect species and multivariate axes based on insect species abundance. See **Table 2** for insect acronyms.

observations for the species composition of flower visitors for the different fields were more similar to each other than what the observations for a given field were across the different years (**Figure 2A**). Also, insect communities in 2017 (before the hurricane events) and 2019 (2 years later) were more similar to each other than what they were to insect communities for all four fields in 2018, 4–6 months after Hurricane Maria hit the island. Kendall correlation analyses showed that the species that most related to the observed variation in the community composition of flower visitors were *Palpada albifrons* (Diptera: Syrphidae), *Palpada vinetorum* (Diptera: Syrphidae), *Apis mellifera* (Hymenoptera: Apidae), *Cochliomyia minima* (Diptera: Calliphoridae), *Psedodorus clavatus* (Diptera: Syrphidae), *Gonia crassicornis* (Diptera: Tachinidae), and *Peckia* sp. (Diptera: Tachinidae) (**Figure 2B** and **Table 1**). Of those, the three most abundant were *Palpada vinetorum*, *Apis mellifera*, and *Cochliomyia minima* (mean observations/field $\geq$ 7.4 individuals/field/tree). Overall, the abundances for *P. vinetorum* and *A. mellifera* were considerably lower in 2018 but that was not the case for *Cochliomyia minima* which became the dominant species with observed abundances that were six times higher than those observed in 2017 (**Figure 3**). In 2019, abundance values for *Cochliomyia minima* had reduced to pre-hurricane levels but those of *A. mellifera* were significantly lower than abundance values in 2017 (**Figure 3**). In contrast, following the decline in 2018, *P. vinetorum* experienced an overall increase in abundance in 2019 and became, once again, the most dominant species by that year. When considering the abundance of dominant species within individual fields, *P. vinetorum* and *A. mellifera* exhibited significant declines in 2018 in all fields but returned to pre-hurricane levels in all fields with the exception of Kent field (**Figure 3** and **Table 2**). In that field, the abundance for

TABLE 1 | Kendall correlation coefficients (r) with ordination axes resulting from Non-metric Multidimensional Scaling (NMDS) analyses for seven species of the communities on four mango fields each containing a different cultivar (Julie, Keitt, Kent, Tommy Atkins).

Species	r (axes 1)	r^2	r (axes 2)	r^2
Palpada vinetorum (Palvin)	−0.495	0.245	−0.874	0.765
Palpada albifrons (Palalb)	−0.652	0.425	−0.517	0.267
Cochliomyia minima (Chomin)	0.94	0.884	0.421	0.177
Psedodorus clavatus (Psecalv)	−0.67	0.449	0.213	0.045
Api mellifera (Apimel)	−0.853	0.727	−0.579	0.335
Gonia crassicornis (Gon)	0.843	0.711	0.301	0.091
Peckia sp. (DipUnk)	0.711	0.506	0.205	0.042

For each species, r^2 *values represent the percentage of variance explained by each axis.*

P. vinetorum increased in 2019 but went above pre-hurricane levels and the abundance for *A. mellifera* did not recover in 2019 (**Figure 3** and **Table 2**). In contrast, the abundance of *C. minima* increased for Keitt, Kent and Tommy Atkins fields in 2018 returning to pre-hurricane levels in 2019 with the Julie field experiencing a decline-recovery pattern between 2018 and 2019 (**Figure 3** and **Table 2**).

Species Richness and Abundance

PERMANOVA detected significant multivariate dissimilarities defined by the total insect diversity and abundance and the abundance of *P. vinetorum*, *A. mellifera*, and *C. minima* explained by differences across years, fields, and their interaction. Fluctuations in species richness and abundance were not consistent across fields. A dendrogram following a cluster analysis using the same variables as in PERMANOVA showed fields within a year indeed clustered together but that relationships among fields within a year were not consistent based on their similarity in species richness, total insect abundance and the abundance of dominant species (**Figure 4**). Repeated measures ANOVA results showed significant differences across years in insect species diversity (i.e., richness) and abundance in only two out of four fields (Kent and Tommy Atkins) but the direction of changes was different for each (**Figures 5A,B** and **Table 3**). On average, values for species richness were lower in 2018 for the Kent area and these returned to pre-hurricane levels by 2019 while in the Tommy Atkin area, trees experienced annual incremental increases in species richness and an increase in insect abundance in 2018 that remained in 2019. The Julie and Keitt fields did not experience significant changes in neither richness nor abundance across years (**Figures 5A,B** and **Table 3**). The variance in species richness also seemed to increase for all fields in 2018 and also remained elevated in 2019 for the Julie, Keitt, and Tommy fields but not for the Kent (**Figure 5B**).

Fruit Production and Crop Yields

With the exception of the Julie field in 2018, crop yield was strongly and positively correlated with fruit yields for all fields and all years (Pearson correlations for fruit production (the number of fruits produced per tree) vs. crop yield; Julie 2018: $r = 0.39$, $P = 0.26$; all other fields: $r = 0.83–0.99$, $p << 0.01$). Results involving crop yield and the number of fruits were always similar in magnitude and direction, thus for the sake of simplicity, we only present results for correlates of plant reproductive output as they relate to crop yield. All fields exhibited declines in the number of inflorescences per tree in 2018 that were followed by significant recoveries in 2019 (**Figure 4C** and **Table 4**). Nevertheless, at the field level, the number of inflorescences per tree during peak flowering was never a good predictor of plant reproductive output (**Table 4**). Instead, plant reproductive output (i.e., crop yield) was positively associated with insect species richness and abundance (**Figure 6**) but the significance and magnitude of these relationships were also influenced by field and sampling year (**Table 4**). In 2017, positive relationships between yield, species richness and insect abundance were strong for all fields (**Figure 6** and **Table 4**). In 2018, following the hurricane events, these associations disappeared for the Julie and Tommy Atkins fields and were only present for the Keitt (only for abundance) and Kent (both for species richness and abundance) although they were weaker than they were in 2017 (**Table 4**). In 2019, relationships between crop yield and species richness and abundance returned for the Julie field and were present in the Keitt field but were absent in the areas with Kent and Tommy Atkins trees. While the Julie and Kent fields had consistent fruit yield values across years, the Kent and Tommy Atkins both experienced an overall reduction in crop yield in 2018 but then recovered to pre-hurricane values in the Kent field and went above pre-hurricane levels in the Tommy Atkins' field in 2019 (**Figure 5D** and **Table 3**). In the Kent field, individuals showed the highest crop yield values of all fields (ave. 407 ± 58.13 kg), followed by the Keitt (ave: 183.25 ± 31.19 kg), Julie (ave: 60.77 ± 7.95 kg), and Tommy Atkins (ave: 77.55 ± 10.63 kg) fields (**Figure 4D**). The abundance of two of the dominant insect species (*P. vinetorum* and *C. minima*) showed positive correlation with reproductive output (crop yields) of mango trees but these relationships were not consistent for all years or fields, nor they were consistent within a single season using conservative alpha values (**Table 5**). With more relaxed alpha values, significant associations between insect abundances and crop yield were encountered in three instances for *P. vinetorum,* two for *C. minima* and none for *A. mellifera*. Using less conservative alpha values, the number of significant correlations increases to eight for *P. vinetorum* (distributed across all years), six for *C. minima* (in 2018 and 2019), and only two for *A. mellifera* (in 2017 and 2019 only in Julie) (**Table 5**).

DISCUSSION

Hurricanes are large-scale weather events with the potential to change insect communities and influence their subsequent dynamics (e.g., Schowalter and Ganio, 1999; McGinley and Willig, 1999; Schowalter et al., 2017). It has been argued that these events may disrupt plant-pollinator interactions with important consequences to food crops (National Research Council et al., 2007; Natalia Escobedo-Kenefic, 2018). While insects carry out most of the pollination function in plants,

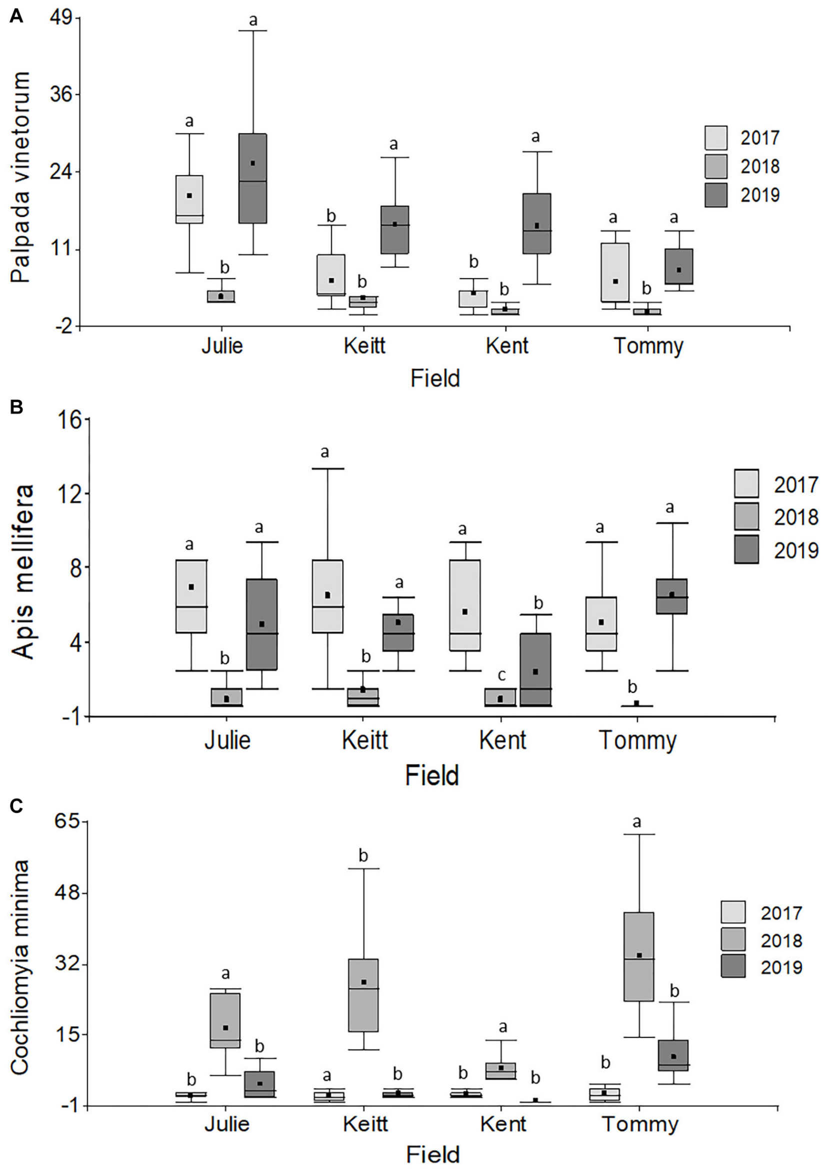

FIGURE 3 | Boxplots illustrating differences in insect abundances per field (Julie, Keitt, Kent, Tommy Atkins) per year for the three most dominant flower visitors. **(A)** *Palpada vinetorum*, **(B)** *Apis mellifera*, **(C)** *Cochliomyia minima.* Repeated Measures analyses performed for each year on each field detected significant differences across years all insect species (**Table 2**). Different letters within a field indicate significant differences among years.

most of what we know about insect responses to hurricane events comes from other functional insect groups (herbivores, predators, omnivores, detritivores; e.g., Torres, 1992; McGinley and Willig, 1999; Schowalter and Ganio, 1999; Koptur et al., 2002; Spiller et al., 2016; Schowalter et al., 2017; Novais et al., 2018). Indeed, studies evaluating the response of insect pollinators to hurricane events have been few and limited in geographic and taxonomic scope which impairs making generalizations on the short- and long-term effects of these interactions (Bronstein and Hossaert-McKey, 1995; Pascarella, 1998; Roubik and Villanueva-Gutierrez, 2009; Landry, 2013). Here we discuss our results on hurricane-induced changes of pollinator communities of *M. indica* on the island of Puerto Rico following the passage of Hurricane Maria to: (1) build knowledge of post-hurricane community dynamics of insect pollinators, and contribute to the extensive literature available for hurricane responses of insect communities; (2) understand the role of diversity and abundance of insect communities vs. the role of individual insect species on the reproduction of a worldwide economically important species, and how these relationships may be influenced by extreme weather events; (3) discuss how this information may inform pollinator management in crop systems in areas where catastrophic weather events such as hurricanes are frequent events.

TABLE 2 | Repeated measures ANOVA analyses testing for annual differences in the average abundance (number of insects observed/tree/day) of dominant insect species for each four fields of *M. indica* each containing a different cultivar.

Species	Field	Year	Mean ± SE	*F*	*P*
Palpada vinetorum	Julie	2017	19.60 ± 2.90	16.7	**0.0001**
		2018	3.00 ± 1.83		
		2019	25.10 ± 2.9		
	Keitt	2017	5.70 ± 1.36	27.1	**0.0001**
		2018	2.90 ± 1.36		
		2019	15.00 ± 1.36		
	Kent	2017	3.70 ± 1.31	44.5	**0.0001**
		2018	0.80 ± 1.31		
		2019	14.90 ± 1.31		
	Tommy Atkins	2017	5.50 ± 1.23	8.64	**0.0023**
		2018	0.40 ± 1.23		
		2019	7.40 ± 1.23		
Apis mellifera	Julie	2017	6.50 ± 0.9	13.4	**0.0003**
		2018	0.40 ± 0.9		
		2019	4.50 ± 0.9		
	Keitt	2017	6.10 ± 0.80	12.5	**0.0004**
		2018	0.90 ± 0.80		
		2019	4.60 ± 0.80		
	Kent	2017	5.20 ± 0.77	11.96	**0.0005**
		2018	0.40 ± 0.77		
		2019	2.70 ± 0.77		
	Tommy Atkins	2017	4.60 ± 0.63	26.75	**0.0001**
		2018	0.40 ± 0.63		
		2019	6.10 ± 0.63		
Cochliomyia minima	Julie	2017	1.3 ± 1.7	38.4	**0.0001**
		2018	18.6 ± 1.7		
		2019	4.1 ± 1.7		
	Keitt	2017	27.60 ± 2.29	43.57	**0.0001**
		2018	1.90 ± 2.29		
		2019	1.10 ± 2.29		
	Kent	2017	1.70 ± 0.55	54.17	**0.0001**
		2018	7.70 ± 0.55		
		2019	0.20 ± 0.55		
	Tommy Atkins	2017	2.00 ± 2.77	35.65	**0.0001**
		2018	33.90 ± 2.77		
		2019	10.20 ± 2.77		

Significant P-values (<0.05) are indicated in bold.

TABLE 3 | Repeated Measures one-way ANOVAs on reproductive traits and insect community traits recorded annually in four fields of *M. indica* between 2017 and 2019.

Trait	Cultivar	*F*	*p*
Number of Inflorescences	Julie	21.89	**0.0001**
	Keitt	36.67	**0.0001**
	Kent	74.17	**0.0001**
	Tommy	21.89	**0.0001**
Yield	Julie	2.53	0.11
	Keitt	0.67	0.53
	Kent	14.80	**0.0002**
	Tommy	17.21	**0.0001**
Total insect abundance	Julie	0.88	0.43
	Keitt	1.07	0.37
	Kent	14.14	**0.0002**
	Tommy	15.12	**0.0001**
Insect species richness	Julie	2.55	0.11
	Keitt	6.52	**0.007**
	Kent	5.57	**0.003**
	Tommy	13.61	**0.0003**

All variables were ln transformed and significant p-values are indicated in bold.

The literature related to post-hurricane dynamics in arthropod communities suggests that while arthropod populations can take different pathways (increase, decrease, or else exhibit complex responses (e.g., Gandhi et al., 2007), under some circumstances these assemblages can be highly resilient and return to pre-hurricane levels in a short period of time (Chen et al., 2020). However, this does not always occur because species responses to hurricanes can depend on the taxon, guild and the environmental context (biotic and abiotic) in which they occur (Schowalter et al., 2017; Novais et al., 2018; Savage et al., 2018). In our system, insect pollinator communities did change after the hurricanes of 2017 but as a whole they were on their way to pre-hurricane levels based on how similar they appeared in multivariate space by 2019. Post-hurricane dynamics of the individual insect pollinator species suggest that these temporal changes in community similarities across years were at least in part driven by changes in their relative abundance and not through the substitution of dominant insect species. At the individual species level, hurricanes clearly reduced the populations of *P. vinetorum* and *A. mellifera* (the two most-dominant species before the hurricanes) but *A. mellifera* abundances was on its way to pre-hurricane levels by 2019 (although not completely) while populations of *P. vinetorum* increased in numbers that year. In contrast, *C. minima* abundances spiked dramatically after the hurricane but decreased to pre-hurricane levels by 2019. A probable explanation for the observed differences in species' post-hurricane abundance dynamics is that hurricane-driven changes in the biotic or abiotic environment influenced the life cycle of different taxa in different ways. For example, many syrphid flies such as *P. vinetorum* have aquatic larvae that are saprophagous and feed on organic matter and microorganisms (Pérez-Bañon et al., 2003; Sánchez-Galván et al., 2017). We know that the 2017 hurricane season resulted in a large accumulation of debris across the island of which 60% was organic (Lugo, 2018). In managed spaces, disposal of this debris was slow and that material might have created ideal habitats for the growth of syrphid larvae during the wet season. Meanwhile, larvae of *Cochliomyia minima* (Calliphoridae) most likely feed on dead carcasses (Yusseff-Vanegas, 2014), which were likely abundant after the hurricane events. Following Hurricane Maria and as a result of canopy cover loss, the island of Puerto Rico exhibited increases in temperatures of up to 4°C in some areas (Lugo, 2018).

Differential taxon responses to hurricane-induced abiotic changes may also explain the observed post-hurricane changes in the dominant taxa. It has been shown that exposure to higher

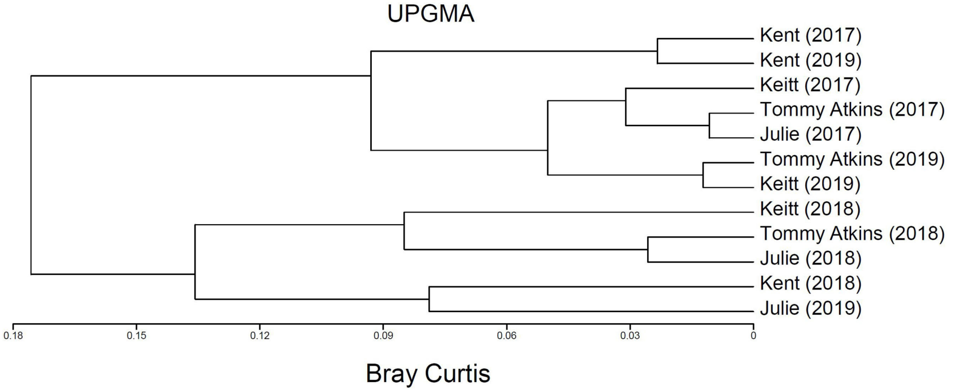

FIGURE 4 | Dendogram depicting multivariate clusters using Bray Curtis dissimilarity index based on total insect species richness, total abundance, and abundances of each of the three dominant insect pollinators of *M. indica* (*Palpada vinetorum, Apis mellifera*, *Cochliomyia minima*) recorded for four fields (Julie, Keitt, Kent, Tommy Atkins) in 2017, 2018, and 2019 at the Juana Diaz Experimental Station in Puerto Rico.

than optimal temperatures in *Apis mellifera* bees may lead to reduced reproductive health in colonies through impaired drone development and reproductive quality, especially if changes are sudden (Rangel and Fisher, 2018 and references therein). High temperature exposures in *Apis mellifera* may also reduce survival and increase oxidative stress in bees (Li et al., 2019). On the other hand, higher temperatures shorten developmental time in Calliphoridae (Bansode et al., 2016), and some species (including some *Cochliomyia*) have high thermal tolerances (42C-53C; Richards et al., 2009). If local *Cochliomyia* have these traits, then it may explain their numerical increases following the hurricanes. Syrphid species, on the other hand, have variable responses (negative and positive) to changes in their abiotic environment but most of this work has come from temperate regions (Radenković et al., 2017; Milić et al., 2019). Clearly more research is needed on the direct and indirect influences of post-hurricane environmental changes to understand the mechanism driving changes of the dominant pollinators of *M. indica*.

Various studies have emphasized the need to ensure diversity and abundance of wild pollinators and the importance of protecting non-bees as a way to enhance pollination services (Blüthgen and Klein, 2011; Albrecht et al., 2012; Thomson, 2019) and crop production (Rader et al., 2013). The protection of the associated crop biodiversity is seen as an important element to ensure food security and sustainability of agricultural systems (FAO, 2018). Our results do show a clear association between pollinator diversity and abundance with fruit production in *M. indica.* However, an important result of this study is that fruit yields were highly reduced in one of the fields evaluated even when pollinator diversity (as expressed by species richness) and abundance increased locally. Therefore, the combined results not only suggest that the relationship between pollinator diversity, abundance and production in this crop is complex, but they also suggest that mechanisms other than changes in pollinators' richness and total abundance themselves will also influence fruit production after hurricanes.

Here we provide several mechanistic hypotheses to explain our results for *M. indica*, some of which may or may not relate to observed changes in pollinator communities. One possibility is that not all *M. indica* pollinators are equally effective and that localized declines in the relative abundances of less abundant pollinators other than the dominant pollinator species studied here led to declines in yields after the hurricane for the Kent and Tommy Atkins fields. Even when all fields experienced declines in *P. vinetorum* and *A. mellifera* (the dominant pollinators) not all fields experienced declines in fruit yields. Moreover, unexpected significant increases in *C. minima* abundance in 2018 for three fields (Keitt, Kent, and Tommy Atkins) did not necessarily translate into concomitant increases in fruit yields for those fields in 2018. Several authors have discussed and tested potential mechanisms in which diversity could stabilize pollination services over time (e.g., Winfree and Kremen, 2009; Mukherjee et al., 2019). These include "density compensation" (i.e., the abundance of one pollinating species increases as a result of reductions in the abundance of another species), "response diversity" (i.e., some pollinating species are increase and others decrease by the same environmental change) and "cross-scale resilience" (i.e., different species are responding to the same environmental stressor at different spatial and temporal scales). While our study cannot be used to discriminate the applicability of these mechanisms, results presented here can be used to evaluate some of the premises of these mechanisms as to what extent these may or may not apply. Our data suggest that the hurricane event indeed influenced dominant species differently (*P. vinetorum and A. mellifera* decreased, while *C. minima* increased) and that these effects were not observed at the same spatial scales (i.e., abundances of *P. vinetorum* and *A mellifera* were reduced for all fields in 2018 but not those of *C. minima* which instead increased for three of the four fields). Also, fruit yield was reduced for Kent and Tommy even when those fields experienced increases in *C. minima* abundance. Those fruit yield patterns do not completely align with a hypothesis of "density compensation" effects by *C. minima* for Kent and Tommy Atkins as their fruit yields decreased

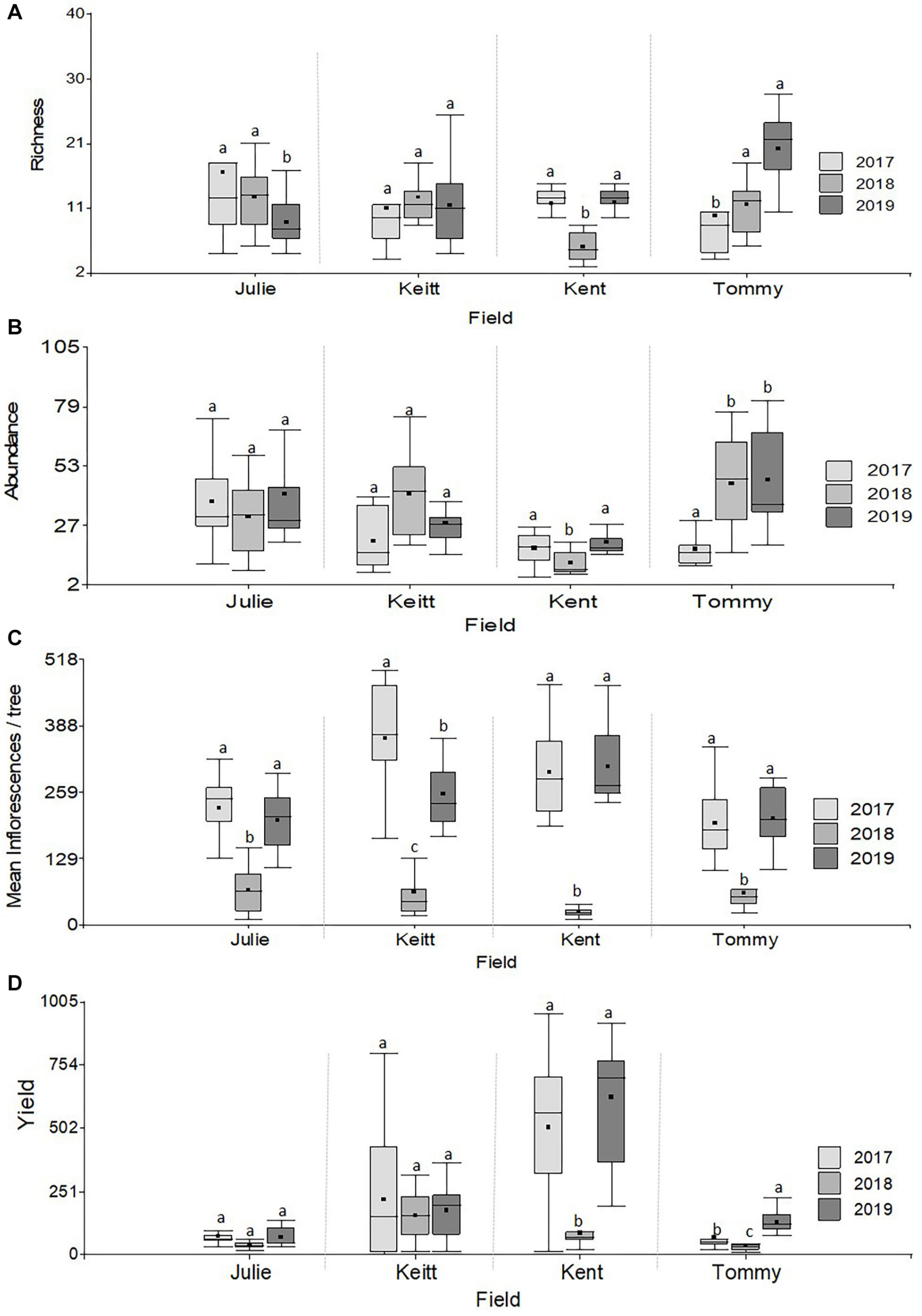

FIGURE 5 | Boxplots illustrating differences in variables related to the state of pollinator communities experienced by trees (insect richness and abundance) and variables related to tree reproductive effort (number of inflorescences and fruit yield) in *M. indica* in 2017, 2018, and 2019. **(A)** Richness, **(B)** Abundance, **(C)** Yield (kg), **(D)** Number of Inflorescences/tree. Different letters within a field indicate significant differences among years following Repeated measures ANOVAs analyses (**Table 3**).

even when abundances for this pollinating species increased for these fields. The fact that even with increases in species richness and abundances in 2018, fruit yields declined in the Tommy Atkins field also suggests that this flower visitor may not be as effective at compensating for losses or reduction in pollination services from other species that may have experienced local reductions in abundance The fact that the Julie field experienced a reduction in all dominant pollinators but not a reduction in fruit yields also suggests the possibility that other less frequent pollinators relative to the ones studied here may be acting as stabilizing influences of fruit yields and in a localized fashion. A long-term study with the generalist non-agricultural species *Scrophularia californica* showed that the relationship between the abundances of the most effective pollinators and reproductive

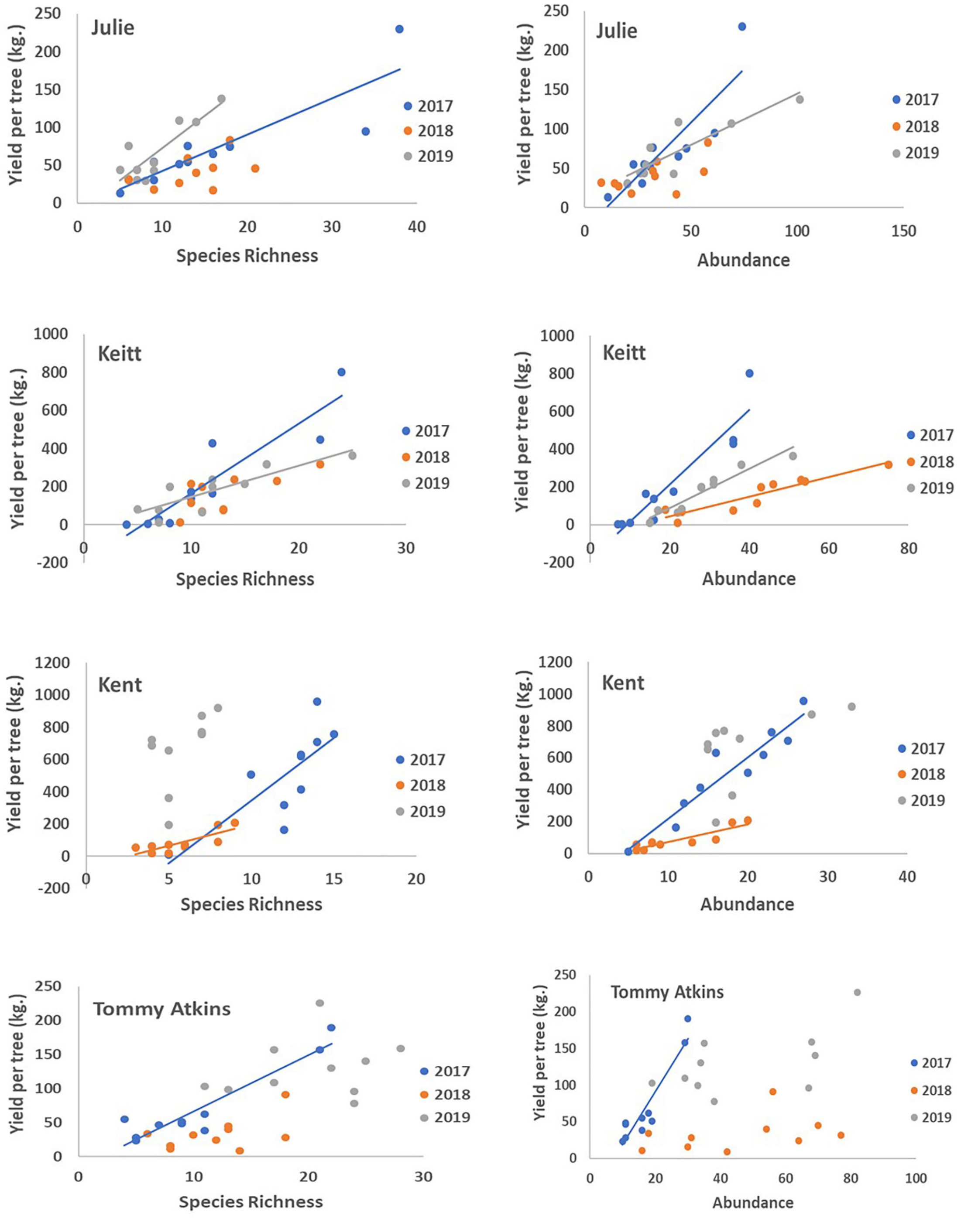

FIGURE 6 | Regressions of fruit yield as a function of global species richness and global insect abundance for four fields (Julie, Keitt, Kent, Tommy Atkins) across the 3 years of the study. For each field GLM analyses detected significant relationships between yield and insect richness and abundance in some years but not others (**Table 4**). Lines represent the line of best fit from significant regressions at $p < 0.05$.

success was non-linear and influenced by variation in spatial and temporal differences in pollinator diversity and abundance (Thomson, 2019). These complex relationships also apply to agricultural systems such as *M. indica*, whose management for pollinator services and diversity, especially following extreme atmospheric events, would therefore require an understanding of plant-pollinator relationships using both community and species-level approaches.

One important result was that correlations between plant yields and the abundances of individual pollinator species were

TABLE 4 | Results for simple regression coefficients from general linear model (GLM) analyses evaluating the variation in reproductive success (plant yield) as a function of insect species richness, insect abundance and the number of inflorescences/trees in four field of *M. indica* in 3 separate years.

Year	Field	Coefficients	Estimate ± SE	Model:AIC	*F*	R^2	*p*
2017	Julie	Richness	1.13 ± 0.15***	13.05	41.9	0.84	**0.0002**
	Julie	Abundance	1.26 ± 0.17***	12.58	44.3	0.85	**0.0002**
	Julie	Inflorescences	0.46 ± 0.24	26.20	1.34	0.28	0.32
		$(\text{Inflorescences})^2$	0.43 ± 0.35				
	Keitt	Richness	3.57 ± 0.52***	35.50	36.64	0.82	**0.0003**
	Keitt	Abundance	3.20 ± 0.40***	32.54	44.3	0.85	**0.0002**
	Keitt	Inflorescences	0.01 ± 0.003**	29.40	3.13	0.47	0.11
	Kent	Richness	3.86 ± 0.47***	22.23	54.3	0.87	**0.0001**
	Kent	Abundance	2.42 ± 0.27***	20.70	64.2	0.89	**0.0001**
	Kent	Inflorescences	0.0008 ± 0.0.0004	39.52	3.05	0.28	0.12
	Tommy Atkins	Richness	0.97 ± 0.21	17.71	17.65	0.69	**0.003**
	Tommy Atkins	Abundance	1.53 ± 0.23***	12.18	36.6	0.82	**0.0003**
	Tommy Atkins	Inflorescences	0.004 ± 0.003	26.17	1.26	0.15	0.27
2018	Julie	Richness	0.45 ± 0.33	21.73	1.49	0.16	0.28
	Julie	Abundance	0.32 ± 0.23	21.59	1.63	0.17	0.24
	Julie	Inflorescences	0.06 ± 0.17	23.31	2.8	0.26	0.13
	Keitt	Richness	2.13 ± 0.87*	32.50	4.63	0.37	0.06
	Keitt	Abundance	1.77 ± 0.42**	26.89	14.15	0.64	**0.006**
	Keitt	Inflorescences	0.71 ± 0.38	34.07	2.8	0.26	0.13
	Kent	Richness	1.47 ± 0.51	26.17	3.6	0.45	**0.03**
	Kent	Abundance	1.45 ± 0.28	19.21	21.31	0.73	**0.002**
	Kent	Inflorescences	0.06 ± 0.17	23.31	1.19	0.27	0.61
	Tommy Atkins	Richness	0.57 ± 0.56	28.55	2.31	0.12	0.26
	Tommy Atkins	Abundance	0.57 ± 0.35	27.50	2.8	0.27	0.13
	Tommy Atkins	Inflorescences	0.09 ± 0.42	29.83	0.04	0.004	0.85
2019	Julie	Richness	1.02 ± 0.31**	17.7	8.89	0.52	**0.02**
	Julie	Abundance	0.91 ± 0.16***	10.26	27.5	0.77	**0.008**
	Julie	Inflorescences	0.70 ± 0.39	23.32	1.62	0.17	0.16
	Keitt	Richness	1.44 ± 0.49*	31.7	6.76	0.46	**0.03**
	Keitt	Abundance	2.53 ± 0.32***	21.4	32.28	0.8	**0.005**
	Keitt	Inflorescences	2.07 ± 1.09	34.77	2.86	0.26	0.13
	Kent	Richness	0.69 ± 0.61	22.58	1.01	0.11	0.34
	Kent	Abundance	0.85 ± 0.53	21.54	2.11	0.21	0.28
	Kent	Inflorescences	−0.70 ± 0.49	23.32	0.02	0.002	0.90
	Tommy Atkins	Richness	0.25 ± 0.32	13.45	0.48	0.06	0.51
	Tommy Atkins	Abundance	0.34 ± 0.18	10.80	3.05	0.28	0.12
	Tommy Atkins	Inflorescences	0.51 ± 0.26	10.80	3.06	0.28	0.12

*Starred values indicate P-values for estimates of regression coefficients: *0.05, **0.01, ***0.001. Models with significant P-values (<0.05) are indicated in bold.*

not consistent across years or fields and that overall *A. mellifera* showed the least number of significant correlations with yield compared to *P. vinetorum* and *C. minima*. Indeed, prior studies have commented on the low attractiveness of mango flowers to honey bees (Free and Williams, 1976) and at least one experimental study that added managed honeybees found no significant increases in fruit production (Carvalheiro et al., 2010). Our results with honeybees are therefore not surprising considering those studies and raises questions about how effective the addition of managed honeybees is as a strategy to improve local pollinator services following hurricane events for crop species like *M. indica*. Indeed, the study by Carvalheiro et al. (2012) suggest that introducing areas of native vegetation to support pollinator services and improve crop production may be a better management strategy worth pursuing for *M. indica* management. On the other hand, the relationships between diversity and abundance of insect pollinators with plant yield were not equally strong for all fields and that post-hurricane changes in these relationships were not expressed equally among fields (**Figure 6**). Each field hosted a different cultivar. Thus, one possibility is that floral characteristics and rewards may differ among cultivars. Mango flowers are minute, and superficially similar among cultivars but could differ in some characteristics which may lead to differential pollinator attraction (Popenoe, 1917). In at least one study, the Keitt cultivar was reported to have low attractiveness relative to Kent and Tommy Atkins (Carvalheiro et al., 2012). We are in the process of analyzing data on floral traits and nectar rewards to address the possibility

TABLE 5 | Pearson correlation coefficients for the number of fruits per tree and the abundance of dominant insect species per year per field.

Insect species	Year	Field	*r*	*p*
Palpada vinetorum	2017	Julie	0.9707	**0.0001**
		Keitt	0.9599	**0.0001**
		Kent	0.3989	0.2534
		Tommy Atkins	0.8236	0.0033
	2018	Julie	0.7684	0.0094
		Keitt	0.337	0.3409
		Kent	N	N
		Tommy Atkins	−0.2129	0.4098
	2019	Julie	0.80547	0.0049
		Keitt	0.9377	**0.0001**
		Kent	0.6623	0.0519
		Tommy Atkins	0.8585	0.0014
Apis mellifera	2017	Julie	0.6939	0.0259
		Keitt	−0.1113	0.7595
		Kent	−0.1125	0.7568
		Tommy Atkins	0.5287	0.1161
	2018	Julie	−0.0381	0.9166
		Keitt	0.4296	0.2152
		Kent	N	N
		Tommy Atkins	−0.3389	0.338
	2019	Julie	0.8174	0.0038
		Keitt	0.6112	0.0604
		Kent	−0.4153	0.2662
		Tommy Atkins	N	N
Cochliomyia minima	2017	Julie	0.0219	0.9520
		Keitt	0.198	0.5834
		Kent	N	N
		Tommy Atkins	0.9138	**0.0002**
	2018	Julie	0.6802	0.0304
		Keitt	0.5535	0.0969
		Kent	0.8618	**0.0013**
		Tommy Atkins	0.7495	0.0126
	2019	Julie	0.6158	0.0579
		Keitt	0.0808	0.8242
		Kent	−0.2806	0.4644
		Tommy Atkins	0.8265	0.0031

Values in bold indicate significant values with a corrected alpha value equal to 0.0002 when corrected for multiple comparisons.

of differences in floral attractiveness or floral resources among cultivars. Likewise, we have ongoing experiments to evaluate potential differences in pollination effectiveness of dominant pollinators of *M. indica* to different cultivars to better understand the role of individual pollinator species and fruit production in this system. A last possibility, and equally likely, is that mango cultivars are in different spatial locations across the station and that landscape differences in insect requirements unknown to us may operate to influence the visitation to individual cultivars. Studies with *M. mangifera* in South Africa have shown that existing flowering resources available within and outside cultivated areas (i.e., natural vegetation) have the potential to influence mango floral visitation by contributing floral resources of shared visitors even outside the mango flowering season (Carvalheiro et al., 2010, 2012; Simba et al., 2018). Carvalheiro et al. (2010), indeed showed that the diversity levels experienced by *M. indica* trees and their fruit yields were dependent on the distance of trees from natural vegetation patches with more diversity and higher yields exhibited by trees that were closer to natural vegetation. There are patches of natural vegetation about 1 km away from the Juana Diaz Experimental Station and visually the matrix of cultivated trees surrounding the fields with the Julie and Keitt cultivars seems to have a more abundant tree cover than trees from the fields with Kent and Tommy Atkins (**Figure 1**).

A second non-mutually exclusive alternative for the unexpected declines in fruit production even when pollinator abundances increased following the hurricane may be related to direct hurricane effects on *M. indica* plants. Hurricane disturbances can damage plants to the point of reducing the resources available to produce flowers and fruits (Rathcke, 2000). A rapid assessment of mango trees at the Juana Diaz Experimental Station following Hurricane Maria, indicated that trees lost between 20 and 90% of their foliage as a result of this event with no tree mortality observed (Cabrera-Asencio, unpublished data). Thus, resource allocation decisions related to the production of leaves vs. fruits may also explain some of the crop yield dynamics but cannot account for all the variation in fruit production. Even when foliage recovery was slow and branch death was still observed in 2019, observed fruit production increased above pre-hurricane levels in 2019 (this study). The lack of tree mortality following hurricane Maria at our site contrasted with a study on mango tree damage in Florida following Hurricane Andrew in 1992 (Department of Health and Human Services, and Department of Agriculture, 1995; Crane and Balerdi, 1996). The Florida mango trees exhibited considerable damage with 58.4% of the trees showing massive damage or mortality which resulted in a 75% reduction in mango production even after 4 years. Furthermore, most mortality occurred in areas where trees were tall and not managed for size. By contrast, trees at the Juana Diaz Experimental Station were managed for size control which may explain different results.

Within season variation in the number of inflorescences did not relate to crop yields and that post hurricane reductions in inflorescence production in mango, while noticeable for all fields, did not necessarily translate into crop yield reductions. Inflorescences of *M. indica* produce numerous and minute unisexual flowers that reach up thousands of flowers like it has been reported for Tommy Atkins (2,238 flowers/inflorescence, Abourayya et al., 2011). It is also widely known that *M. indica* in cultivation produces many more flowers than its sets fruits (fruit sets ∼ 10%; Shu, 2009). Thus, one unexplored possibility is that even with the reductions of inflorescences and pollinators, following the hurricanes, the resulting flower:pollinator ratios still allowed to maximize fruit production in some cultivars. Also, the ratio of hermaphroditic to male flowers in andromonoecious plant systems like *M. indica* can vary in response to environmental stressors (Geetha et al., 2016) in ways that may reflect an optimal use of available resources to reproduction. Thus, another unexplored and not mutually exclusive possibility is that some cultivars of *M. indica* are also able to adjust floral resources in such a way that resources are used more efficiently toward producing fruits. These ideas would need to be explored thoroughly to understand how hurricane changes in floral resources and pollinators may ultimately influence reproduction in *M. indica*.

Fruit yields in this economically important species can bounce back (under some conditions) in less than 2 years. In the case of *M. indica* and assuming low mortality, two conditions may potentiate this recovery. The majority of insects visiting the *M. indica* cultivars are native to the Caribbean region and probably have, through their evolutionary history, developed adaptations that could make them highly resilient to these systems regardless of taxa. Second, the effects of hurricane-related changes in pollinator faunas on plant reproduction (regardless of the plant system) may depend whether or not pollination systems are generalized or specialized (Dalsgaard et al., 2009) or whether or not plant species have alternative mechanisms of plant reproductive assurance (Jones et al., 2001; Rivera-Marchand and Ackerman, 2006; Pérez et al., 2018). While most cultivars of *M. indica* depend on animal pollination to set fruit (Anderson et al., 1982; De Siqueira et al., 2008; Fajardo et al., 2008; Corredor and García, 2011; Kumar et al., 2012), it is apparent that globally mangos have a highly generalized pollination system, a condition that is likely favorable where extreme weather events are common such as in the Caribbean. Plant reproductive ecology theory states that the capacity to be pollinated by a large diversity of insects may allow for functional redundancy through shifts in dominant pollinator species, allowing for reproduction to occur in highly variable environments (Waser et al., 1996). A generalist pollination system may explain, at least in part, the naturalization and success of this crop in many areas of Latin America and the Caribbean. Whether such success may continue in the face of climatic change and expected increases in extreme weather events depends on long-term monitoring schemes. The use of information on pollinator diversity and abundance in this system for successful agriculture would certainly require more in-depth information on the role of global insect diversity and abundance vs. the role of individual species themselves.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

IC-A and EM-A conceived the study, designed the experiments, and co-wrote the manuscript. IC-A conducted the field research and observations, collected the data, and performed the statistical analysis. EM-A contributed to the data analysis and manuscript development strategy. All authors reviewed the manuscript, contributed to the article, and approved the submitted version.

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REFERENCES

Abourayya, M. S., Kassim, N. E., El-Sheikh, M. H., and Rakha, A. M. (2011). Comparative study between inflorescences characteristics, pollen viability, germination, and dimensions of Tommy Atkins, Kent, and Keitt mango cultivars. *Life Sci. J.* 8, 100–105.

Aizen, M. A., Aguiar, S., Biesmeijer, J. C., Garibaldi, L. A., Inouye, D. W., Jung, C., et al. (2019). Global agricultural productivity is threatened by increasing pollinator dependence without a parallel increase in crop diversification. *Global Change Biol.* 25, 3516–3527. doi: 10.1111/gcb.14736

Albrecht, M., Schmid, B., Hautier, Y., and Müller, C. B. (2012). Diverse pollinator communities enhance plant reproductive success. *Proc. R. Soc. B: Biol. Sci.* 279, 4845–4852. doi: 10.1098/rspb.2012.1621

Anderson, D. L., Sedgley, M., Short, J. R. T., and Allwood, A. J. (1982). Insect pollination of mango in Northern Australia. *Aust. J. Agric. Res.* 33, 541–548. doi: 10.1071/ar9820541

Anderson, M. J. (2014). *Permutational Multivariate Analysis of Variance (PERMANOVA)*. Hoboken, NJ: Wiley.

Anselin, L., Syabri, I., and Kho, Y. (2006). GeoDa : an introduction to spatial data analysis. *Geograph. Anal.* 38, 5–22. doi: 10.1111/j.0016-7363.2005.00671.x

Bansode, S. A., More, V. R., and Zambare, S. P. (2016). Effect of different constant temperature on the life cycle of a fly of forensic importance Lucilia cuprina. *Entomol. Ornithol. Herpetol.* 5, 2161–2983.

Bartomeus, I., Ascher, J. S., Gibbs, J., Danforth, B. N., Wagner, D. L., Hedtke, S. M., et al. (2013). Historical changes in northeastern US bee pollinators related to shared ecological traits. *Proc. Natl. Acad. Sci. U S A.* 110, 4656–4660. doi: 10.1073/pnas.1218503110

Beinroth, F. H., Engel, R. J., Lugo, J. L., Santiago, C. L., Ríos, S., and Brannon, G. (2003). Updated taxonomic classification of the soils of puerto rico, 2002. *Agr. Exp. de P.R.* 303:73.

Blüthgen, N., and Klein, A. M. (2011). Functional complementarity and specialisation: the role of biodiversity in plant–pollinator interactions. *Basic Appl. Ecol.* 12, 282–291. doi: 10.1016/j.baae.2010.11.001

Bronstein, J. L., and Hossaert-McKey, M. (1995). Hurricane Andrew and a Florida fig pollination mutualism: resilience of an obligate interaction. *Biotropica* 27, 373–381. doi: 10.2307/2388922

Burnham, K. P., and Anderson, D. R. (2002). *Model Selection and Multimodel Inference: a Practical Information-theoretic Approach*, 2nd Edn. New York, NY: Springer-Verlag.

Cameron, S. A., Lozier, J. D., Strange, J. P., Koch, J. B., Cordes, N., Solter, L. F., et al. (2011). Patterns of widespread decline in North American bumble bees. *Proc. Natl. Acad. Sci. U S A.* 108, 662–667. doi: 10.1073/pnas.1014743108

Campbell, R. J. (1992). *Mangos: A Guide to Mangos in Florida (No. Ph0756)*. Coral Gables: FL: Fairchild Tropical Gardens.

Carvalheiro, L. G., Seymour, C. L., Nicolson, S. W., and Veldtman, R. (2012). Creating patches of native flowers facilitates crop pollination in large agricultural fields: mango as a case study. *J. Appl. Ecol.* 49, 1373–1383. doi: 10.1111/j.1365-2664.2012.02217.x

Carvalheiro, L. G., Seymour, C. L., Veldtman, R., and Nicolson, S. W. (2010). Pollination services decline with distance from natural habitat even in biodiversity-rich areas. *J. Appl. Ecol.* 47, 810–820. doi: 10.1111/j.1365-2664.2010.01829.x

Cavanaugh, J. E. (1997). Unifying the derivations for the Akaike and corrected Akaike information criteria. *Statist. Probabil. Lett.* 33, 201–208. doi: 10.1016/s0167-7152(96)00128-9

Central America Data (2016). *Más Países Exportan Mangó a EE.UU*. Available online at: https://www.centralamericadata.com/es/article/home/Ms_pases_exportan_mango_a_EEUU (accessed February 5, 2021).

Chen, X., Adams, B. J., Platt, W. J., and Hooper-Bùi, L. M. (2020). Effects of a tropical cyclone on salt marsh insect communities and post-cyclone reassembly processes. *Ecography* 43, 843–847.

Clarke, K. R. (1993). Non-parametric multivariate analysis of changes in community structure. *Aus. J. Ecol.* 18, 117–143. doi: 10.1111/j.1442-9993.1993.tb00438.x

Coetzer, L. A., Oosthuye, S. A., Wishart, D. L., and Robbertse, P. J. (1995). Influence of pruning on the flower sex ratio in some mango cultivars. s.a. mango growers assoc. *Yearbook* 15, 26–30.

Convention on Biological Diversity (2018). *Convention and Sustainable Use of Pollinators*. Rio de Janeiro: Convention on Biological Diversity.

Corredor, J. P., and García, L. J. (2011). Fenología, biología floral y visitantes florales en los cultivares de mango (Mangifera indica) Hilacha y Tommy Atkins en el valle del alto Magdalena (Colombia). *Corpoica. Tecnol. Agropecu.* 12, 21–32. doi: 10.21930/rcta.vol12_num1_art:212

Crane, J. H., and Balerdi, C. F. (1996). Effect of hurricane andrew on mango trees in Florida and their recovery. *Acta Hort.* 455, 323–330. doi: 10.17660/actahortic.1997.455.42

Dag, A., and Gazit, S. (2000). Mango pollinators in Israel. *J. Appl.Hort.* 2, 39–43. doi: 10.37855/jah.2000.v02i01.12

Dalsgaard, B., González, A. M. M., Olesen, J. M., Ollerton, J., Timmermann, A., Andersen, L. H., et al. (2009). Plant–hummingbird interactions in the West Indies: floral specialisation gradients associated with environment and hummingbird size. *Oecologia* 159, 757–766. doi: 10.1007/s00442-008-1255-z

De Siqueira, K. M. M., Kiill, L. H. P., Martins, C. F., Lemos, I. B., Monteiro, S. P., and Feirosa, E. A. (2008). Comparative study of pollination of *Mangifera indica* L. in conventional and organic crops in the region of the Subme'dio Sao Francisco valley. *Revista Brasileira de Fruticultura* 30, 303–310.

Department of Health and Human Services, and Department of Agriculture (1995). *Ten-year Comprehensive Plan for the National Nutrition Monitoring and Related Research Program*. Washington, D.C: Department of Health and Human Services.

Di Rienzo, J. A., Casanoves, F., Balzarini, M., Gonzalez, L., Tablada, M., and Robledo, C. W. (2018). *InfoStat. Software Estadístico*. Córdoba: Universidad Nacional de Córdoba.

Dormann, F. C., McPherson, J. M., Araújo, B. M., Bivand, R., Bolliger, J., Carl, G., et al. (2007). Methods to account for spatial autocorrelation in the analysis of species distributional data: a review. *Ecography* 30, 609–628. doi: 10.1111/j.2007.0906-7590.05171.x

Environmental Protection Agency [EPA] (2016). *Climate change indicators in the United States, 2016*, 4th Edn. Washington, D.C: Environmental Protection Agency.

Ewel, J. J., and Whitmore, J. L. (1973). *The Ecological Life Zones of Puerto Rico and the US Virgin Islands*. Washington, D.C: USDA. USDA Forest Service, Institute of Tropical Forestry, Research Paper ITF-018, 18.

Fain, S. J., Quiñones, M., Álvarez-Berríos, N. L., Parés-Ramos, I. K., and Gould, W. A. (2018). Climate change and coffee: assessing vulnerability by modeling future climate suitability in the Caribbean island of Puerto Rico. *Clim. Change* 146, 175–186. doi: 10.1007/s10584-017-1949-5

Fajardo, A. C., Medina, R. J., Opina, O. S., and Cervancia, C. R. (2008). Insect pollinators and floral visitors of mango (Mangifera indica L. C.V. Carabao). *Phil. Agricult. Sci.* 91, 372–382.

FAO (2018). *AGP-Pollination*. Rome: FAO.

FAOSTAT (2000). *FAOSTAT Database Result (2000)*. Rome: FAOSTAT.

Feng, Y., Negron-Juarez, R. I., Patricola, C. M., Collins, W. D., Uriarte, M., Hall, J. S., et al. (2018). Rapid remote sensing assessment of impacts from Hurricane Maria on forests of Puerto Rico. *PeerJ Inc.* 6:e26597v1.

Free, J. B., and Williams, I. H. (1976). Insect pollination of *Anacardium occidentale* L., *Mangifera indica* L., *Blighia sapida* koening and persea americana mill. *Trop. Agric.* 53, 125–139.

Galán, S. V. (2009). El cultivo del mango. Situación Botanica 2a. Ediciones Mundi-Prensa Libros. México. 33–35

Gandhi, K. J., Gilmore, D. W., Katovich, S. A., Mattson, W. J., Spence, J. R., and Seybold, S. J. (2007). Physical effects of weather events on the abundance and diversity of insects in North American forests. *Environ. Rev.* 15, 113–152. doi: 10.1139/a07-003

Garibaldi, L. A., Steffan-Dewenter, I., Winfree, R., Aizen, M. A., Bommarco, R., Cunningham, S. A., et al. (2013). Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science* 339, 1608–1611. doi: 10.1126/science.1230200

Garratt, M. P. D., Breeze, T. D., Jenner, N., Polce, C., Biesmeijer, J. C., and Potts, S. G. (2014). Avoiding a bad apple: insect pollination enhances fruit quality and economic value. *Agricult. Ecosystems Environ.* 184, 34–40. doi: 10.1016/j.agee.2013.10.032

Geetha, G. A., Shivashankara, K. S., and Reddy, Y. T. N. (2016). Varietal variations in temperature response for hermaphrodite flower production and fruit set in mango (*Mangifera indica L*). *South African J. Botany* 106, 196–203. doi: 10.1016/j.sajb.2016.07.011

Goguey, T. (1997). Architectural Approach of the mechanisms of canopy growth and flowering of mango trees. *Acta Horticult.* 455, 124–134. doi: 10.17660/actahortic.1997.455.17

Gómez, J. M., Bosch, J., Perfectti, F., Fernández, J., and Abdelaziz, M. (2007). Pollinator diversity affects plant reproduction and recruitment: the tradeoffs of generalization. *Oecologia* 153, 597–605. doi: 10.1007/s00442-007-0758-3

González-Varo, J. P., Biesmeijer, J. C., Bommarco, R., Potts, S. G., Schweiger, O., Smith, H. G., et al. (2013). Combined effects of global change pressures on animal-mediated pollination. *Trends Ecol. Evol.* 28, 524–530. doi: 10.1016/j.tree.2013.05.008

Gould, W., Fain, S. J., Pares, I. K., McGinley, K., Perry, A., and Steele, R. F. (2015). *Caribbean Regional Climate Sub Hub Assessment of Climate Change Vulnerability and adaptation and Mitigation Strategies*. Washington, D.C: United States Department of Agriculture.

Hammer, Ø, Harper, D. A., and Ryan, P. D. (2001). PAST: paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* 4:9.

Harmsen, E. W., Tosado, P., and Mecikalski, J. (2014). Calibration of selected pyrometers and satellite derived solar radiation in Puerto Rico. *Int. J. Renew. Energy Technol.* 5, 43–54. doi: 10.1504/IJRET.2014.059660

Hsin Sung, I., Ming-Yin, L. G., Chin-Hsing, C., Ann-Shiou, C., and Wen-Shyong, C. (2006). Pollinators and their behaviors on mango flowers in Southern Taiwan. *Formosan Entomol.* 26, 161–170.

Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services [IPBES] (2016). *Summary for Policymakers of the Assessment Report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on Pollinators, Pollination and Food Production.* Bonn: IPBES, 1–30.

Jevanandam, N., Goh, A. G., and Corlett, R. T. (2013). Climate warming and the potential extinction of fig wasps, the obligate pollinators of figs. *Biol. Lett.* 9:20130041. doi: 10.1098/rsbl.2013.0041

Jiron, L. F., and Hedström, I. (1985). Pollination ecology of mango (*Mangifera indica L.*) (Anacardiaceae) in the neotropic region. *Turrialba* 35, 269–277.

Jones, K. E., Barlow, K. E., Vaughan, N., Rodríguez-Durán, A., and Gannon, M. R. (2001). Short-term impacts of extreme environmental disturbance on the bats of Puerto Rico. *Animal Conserv. Forum* 4, 59–66. doi: 10.1017/s1367943001001068

Klein, A.-M., and Bernard, E. (2007). Vaissiere, James H Cane, ingolf steffan-dewenter, saul a cunningham, claire kremen, and teja tscharntke. importance of pollinators in changing landscapes for world crops. *Proc. R. Soc. B-Biol. Sci.* 274, 303–313. doi: 10.1098/rspb.2006.3721

Koptur, S., Rodriguez, M. C., Oberbauer, S. F., Weekley, C., and Herndon, A. (2002). Herbivore-free time? damage to new leaves of woody plants after hurricane andrew 1. *Biotropica* 34, 547–554. doi: 10.1111/j.1744-7429.2002.tb00573.x

Kovach, W. L. (2007). *MVSP - A MultiVariate Statistical Package for Windows, ver. 3.1*. Pentraeth: Kovach Computing Services.

Kumar, P., Baskaran, S., Sundaravadivelan, C., Kuberan, T., and Anburaj, J. (2012). Influence of environmental factor son insect pollination activity of mangifera indica linn. *Asian J. Plant Sci. Res.* 2, 692–698.

Landry, C. L. (2013). Changes in pollinator assemblages following hurricanes affect the mating system of *Laguncularia racemosa* (Combretaceae) in Florida, USA. *J. Trop. Ecol.* 29, 209–216. doi: 10.1017/s0266467413000266

Li, X., Ma, W., Shen, J., Long, D., Feng, Y., Su, W., et al. (2019). Tolerance and response of two honeybee species *Apis cerana* and *Apis mellifera* to high temperature and relative humidity. *PLoS One* 14:e0217921. doi: 10.1371/journal.pone.0217921

Losey, J. E., and Vaughan, M. (2006). The economic value of ecological services provided by insects. *Bioscience* 56, 311–323. doi: 10.1641/0006-3568(2006)56[311:tevoes]2.0.co;2

Lugo, A. E. (2018). *Social-Ecological-Technological Effects of Hurricane María on Puerto Rico: Planning for Resilience under Extreme Events*. Berlin: Springer.

Marquis, R. J., Lill, J. T., Forkner, R. E., Le Corff, J., Landosky, J. M., and Whitfield, J. B. (2019). Declines and resilience of communities of leaf chewing insects on missouri oaks following spring frost and summer drought. *Front. Ecol. Evol.* 7:396. doi: 10.3389/fevo.2019.00396

McCune, B., and Grace, J. (2002). *Analysis of Ecological Communities. MJM Software Design*. Oregon, OR: Gleneden beach.

McCune, B., and Mefford, M. J. (2006). *PC-ORD. Multivariate Analysis of Ecological Data, Version 5.0 for Windows. MJM Software*. Oregon, OR: Gleneden Beach.

Memmott, J., Craze, P. G., Waser, N. M., and Price, M. V. (2007). Global warming and the disruption of plant-pollinator interactions. *Ecol. Lett.* 10, 710–717. doi: 10.1111/j.1461-0248.2007.01061.x

McGinley, M. A., and Willig, M. R. (1999). "The response of animals to disturbance and their roles in patch generation," in *Ecosystems of Disturbed Ground,* ed. L. Walker (Amsterdam: Elsevier), 633–657.

Milić, D., Radenković, S., Radišić, D., Andrić, A., Nikolić, T., and Vujić, A. (2019). Stability and changes in the distribution of *Pipiza hoverflies* (Diptera, Syrphidae) in Europe under projected future climate conditions. *PLoS One* 14:e0221934. doi: 10.1371/journal.pone.0221934

Morton, J. (1987). *Mango in Fruits of Warm Climates*. Miami, FL: J F. Morton.

Mukherjee, R., Deb, R., and Devy, S. M. (2019). Diversity matters: effects of density compensation in pollination service during rainfall shift. *Ecol. Evol.* 9, 9701–9711. doi: 10.1002/ece3.5500

Natalia Escobedo-Kenefic (2018). *Background Document for the Biodiversity and Ecosystem Services Network (BES-Net) Caribbean Regional Trialogue on Pollinators, Food Security and Climate Resilience. Biodiversity and Ecosystem Services Network*. Available online at: https://www.besnet.world/sites/default/files//event/document/Background%20document%20online_English.pdf (accessed February 5, 2021).

National Research Council, Board on Agriculture, Committee on the Nutrient Requirements of Small Ruminants, and Division on Earth and Life Studies (2007). *Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids*. Washington, D.C: National Research Council.

Novais, S., Macedo-Reis, L. E., Cristóbal-Pérez, E. J., Sánchez-Montoya, G., Janda, M., Neves, F., et al. (2018). Positive effects of the catastrophic Hurricane Patricia on insect communities. *Sci. Rep.* 8:15042.

Pascarella, J. B. (1998). Hurricane disturbance, plant-animal interactions, and the reproductive success of a tropical shrub 1. *Biotropica* 30, 416–424. doi: 10.1111/j.1744-7429.1998.tb00075.x

Pérez, M. E., Meléndez-Ackerman, E. J., and Monsegur-Rivera, O. A. (2018). Breeding system and pollination of *Gesneria pauciflora* (Gesneriaceae), a threatened Caribbean species. *Flora* 242, 8–15. doi: 10.1016/j.flora.2018.02.009

Pérez-Bañon, C., Rotheray, G., Hancock, G., Marcos-García, M. A., and Zumbado, M. A. (2003). Immature stages and breeding sites of some neotropical saprophagous syrphids (Diptera: Syrphidae). *Annals Entomol. Soc. Am.* 96, 458–471. doi: 10.1603/0013-8746(2003)096[0458:isabso]2.0.co;2

Popenoe, W. (1917). *The Pollination of the Mango (No. 542)*. Washington, D.C: US Department of Agriculture.

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., and Kunin, W. E. (2010a). Global pollinator declines: trends, impacts and drivers. *Trends Ecol. Evol.* 25, 345–353. doi: 10.1016/j.tree.2010.01.007

Potts, S. G., Roberts, S. P. M., Dean, R., Marris, G., Brown, M. A., Jones, R., et al. (2010b). Declines of managed honey bees and beekeepers in Europe. *J. Apicult. Res.* 49, 15–22. doi: 10.3896/ibra.1.49.1.02

Radenković, S., Schweiger, O., Milić, D., Harpke, A., and Vujić, A. (2017). Living on the edge: forecasting the trends in abundance and distribution of the largest hoverfly genus (Diptera: Syrphidae) on the Balkan Peninsula under future climate change. *Biol. Conserv.* 212, 216–229. doi: 10.1016/j.biocon.2017.06.026

Rader, R., Bartomeus, I., Garibaldi, L. A., Garratt, M. P., Howlett, B. G., Winfree, R., et al. (2016). Non-bee insects are important contributors to global crop pollination. *Proc. Natl. Acad. Sci. U S A.* 113, 146–151.

Rader, R., Edwards, W., Westcott, D. A., Cunningham, S. A., and Howlett, B. G. (2013). Diurnal effectiveness of pollination by bees and flies in agricultural *Brassica rapa*: implications for ecosystem resilience. *Basic Appl. Ecol.* 14, 20–27. doi: 10.1016/j.baae.2012.10.011

Ramirez, F., and Davenport, T. L. (2010). Mango (Mangifera indica L.) flowering physiology. *Sci. Horticult.* 126, 65–72. doi: 10.1016/j.scienta.2010.06.024

Ramírez, F., and Davenport, T. L. (2016). Mango (Mangifera indica L.) pollination: a review. *Sci. Horticult.* 203, 158–168. doi: 10.1016/j.scienta.2016.03.011

Rangel, J., and Fisher, A. (2018). Factors affecting the reproductive health of honey bee (*Apis mellifera*) drones—a review. *Apidologie* 50, 759–778. doi: 10.1007/s13592-019-00684-x

Rathcke, B. J. (2000). Hurricane causes resource and pollination limitation of fruit set in a bird-pollinated shrub. *Ecology* 81, 1951–1958. doi: 10.1890/0012-9658(2000)081[1951:hcrapl]2.0.co;2

Richards, C. S., Price, B. W., and Villet, M. H. (2009). Thermal ecophysiology of seven carrion-feeding blowflies in Southern Africa. *Entomol. Exp. et Appl.* 131, 11–19. doi: 10.1111/j.1570-7458.2009.00824.x

Rivera-Marchand, B., and Ackerman, J. D. (2006). Bat pollination breakdown in the Caribbean columnar cactus *Pilosocereus royenii*. *Biotropica* 38, 635–642. doi: 10.1111/j.1744-7429.2006.00179.x

Rogers, S. R., Tarpy, D. R., and Burrack, H. J. (2013). Multiple criteria for evaluating pollinator performance in highbush blueberry (Ericales: Ericaceae) agroecosystems. *Environ. Entomol.* 42, 1201–1209. doi: 10.1603/en12303

Roubik, D. W., and Villanueva-Gutierrez, R. (2009). Invasive Africanized honey bee impact on native solitary bees: a pollen resource and trap nest analysis. *Biol. J. Linnean Soc.* 98, 152–160. doi: 10.1111/j.1095-8312.2009.01275.x

Sánchez-Galván, I. R., Ferrer, J., Galante, E., and Marcos-García, M. A. (2017). Bacteria and hoverflies (Diptera: Syrphidae) in tree hollows from the Iberian mediterranean forest. *Environ. Entomol.* 46, 137–142.

SAS Institute Inc (2019). *Certified Professional Prep Guide: Advanced Programming Using SAS 9.4.* Cary, NC

Savage, A. M., Youngsteadt, E., Ernst, A. F., Powers, S. A., Dunn, R. R., and Frank, S. D. (2018). Homogenizing an urban habitat mosaic: arthropod diversity declines in New York City parks after SuperStorm Sandy. *Ecol. Appl.* 28, 225–236. doi: 10.1002/eap.1643

Schowalter, T. D. (2012). Insect responses to major landscape-level disturbance. *Annual Rev. Entomol.* 57, 1–20. doi: 10.1146/annurev-ento-120710-100610

Schowalter, T. D., and Ganio, L. M. (1999). Invertebrate communities in a tropical rain forest canopy in Puerto Rico following Hurricane Hugo. *Ecol. Entomol.* 24, 191–201. doi: 10.1046/j.1365-2311.1999.00186.x

Schowalter, T. D., Willig, M. R., and Presley, S. J. (2017). Post-hurricane successional dynamics in abundance and diversity of canopy arthropods in a tropical rainforest. *Environ. Entomol.* 46, 11–20.

Shu, Z. H. (2009). Sex distribution, sex ratio and natural pollination percentage of mango (*Mangifera indica* L.). *Acta Hortic.* 820, 205–212. doi10.17660/ActaHortic.2009.820.22

Simba, L. D., Foord, S. H., Thébault, E., van Veen, F. F., Joseph, G. S., and Seymour, C. L. (2018). Indirect interactions between crops and natural vegetation through flower visitors: the importance of temporal as well as spatial spillover. *Agricult. Ecosystems Environ.* 253, 148–156. doi: 10.1016/j.agee.2017.11.002

Singh, G. (1988). Insect Pollinators of mango and their role in fruit setting. *Acta Horticult.* 231, 629–632. doi: 10.17660/actahortic.1989.231.22

Spiller, D. A., Schoener, T. W., and Piovia-Scott, J. (2016). Predators suppress herbivore outbreaks and enhance plant recovery following hurricanes. *Ecology* 97:10.

Thomson, D. M. (2019). Effects of long-term variation in pollinator abundance and diversity on reproduction of a generalist plant. *J. Ecol.* 107, 491–502. doi: 10.1111/1365-2745.13055

Torres, J. A. (1992). Lepidoptera outbreaks in response to successional changes after the passage of Hurricane Hugo in Puerto Rico. *J. Trop. Ecol.* 8, 285–298. doi: 10.1017/s0266467400006544

U.S. Forest Service (2018). *In the Aftermath of Hurricane Maria- Part 3: El Yunque National Forest*. Available online at: https://www.fs.fed.us/inside-fs/aftermath-hurricane-maria-part-3-el-yunque-national-forest (accessed February 5, 2021).

Villanueva-Gutierrez, R., Roubik, D. W., and Colli-Ucán, W. (2005). Extinction of *Melipona beecheii* and traditional beekeeping in the *Yucatán peninsula*. *Bee World* 86, 35–41.

Waser, N. M., Chittka, L., Price, M. V., Williams, N. M., and Ollerton, J. (1996). Generalization in pollination systems, and why it matters. *Ecology* 77, 1043–1060. doi: 10.2307/2265575

Winfree, R., and Kremen, C. (2009). Are ecosystem services stabilized by differences among species? a test using crop pollination. *Proc. Biol. Sci.* 276, 229–237. doi: 10.1098/rspb.2008.0709

Wongsiri, S., and Chen, P. P. (1995). Effects of agricultural development on honey bees in thailand. *Bee World* 76, 3–5.

Yusseff-Vanegas, S. (2014). Description of third instars of *Cochliomyia minima* (Diptera: Calliphoridae) from the West Indies, and updated identification key. *J. Med. Entomol.* 51, 1051–1056. doi: 10.1603/me13088

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Invasive Species in Puerto Rico: The View From El Yunque

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Native flora and fauna of Puerto Rico have a long biogeographic connection to South America. Theory and empirical evidence suggest that islands, particularly those distantly isolated from the mainland, should be more susceptible to naturalizations and invasions of non-native species than continental areas. Anthropogenic disturbances can facilitate accidental and deliberate introductions of non-native species. In this study, we asked: What is the current status of introduced species within El Yunque National Forest (EYNF), the largest and most well-conserved forest area of Puerto Rico? To address this question, we reviewed the literature and surveyed local experts to identify introduced plant and animal taxa that are behaving as invaders within EYNF. We hypothesized that well-conserved forest areas within EYNF would be more resistant to invasions than disturbed areas along roads and ruderal areas with a long history of human activity. We found that there is only partial evidence that supports our hypothesis and this evidence is strongest in vascular plants, but not for the other taxonomic groups analyzed. Our combined results showed that currently the more ubiquitous invasive species in EYNF include some mammals (feral cat, rat, and mongoose) and some invertebrates (earthworms, mosquito, and Africanized honeybee). For many taxa, there is little information to thoroughly test our hypothesis, and thus more detailed surveys of the status of non-native and invasive species in EYNF are needed.

Keywords: alien biodiversity, biotic resistance, introduced animals, island biogeography, Luquillo Experimental Forest, non-native plants, tropical montane forest

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INTRODUCTION

Islands are isolated land masses that frequently exhibit simplified ecological systems containing locally adapted and endemic species, often with small population sizes, low reproductive rates, and a lack of predator defenses compared with mainland counterparts (Wilson and MacArthur, 1967; Moser et al., 2018). These attributes make island ecosystems more susceptible than mainland ecosystems to human-related impacts, such as the introduction and establishment of non-native species. The theory of island biogeography (Wilson and MacArthur, 1967) states that isolation and island size are critical factors determining the number of species that can colonize and establish on island ecosystems. Depending on species' vagility, islands near the mainland may have similar native species composition and ecological characteristics and higher species richness than remote islands. Thus, islands with a biogeographic history of lying near continental areas may be more resistant to the establishment of new species than those isolated far from mainland

(Moser et al., 2018). Islands across the Caribbean show strong species affinities with both North and South America because of their shared biogeographic history, which largely results from their proximity to these two continental regions (Roncal et al., 2020). Therefore, we might expect Caribbean islands to be more diverse and therefore more resistant to the establishment of introduced species, including those that are potentially invasive (i.e., species spreading rapidly into new areas; terminology follows Blackburn et al., 2011) than more isolated islands in ocean basins.

Humans are the ultimate ecosystem engineers, altering habitats for shelter, provision, and resource acquisition. Via their activities, they often introduce new species to areas they colonize, either intentionally for provision or ornamentation or unintentionally by bringing along parasites or commensals. Thus, isolation is effectively reduced. Studies have shown that in addition to isolation and island area (typical factors of the island biogeography theory), factors such as levels of anthropogenic disturbance, human activities (e.g., economic development, human population size, trade and transportation rates and pathways), and propagule pressure are key drivers explaining the diversity of introduced species and invasion success on islands (e.g., Gallardo et al., 2015). Human-mediated introductions of non-native species to islands may lead not only to increases in the number and distribution of species that may colonize islands but also to the displacement and/or extinction of native species. In this regard, human-related activities may lead to a breakdown of the "classical" biogeographic theory and may "redefine" species diversity on islands (Capinha et al., 2015; Blackburn et al., 2016; Rojas-Sandoval et al., 2020).

Caribbean islands have a long history of humans intentionally or accidentally introducing non-native species, and a subset of these have become invasive (e.g., Kairo et al., 2003; Reynolds and Niemiller, 2010; Wilson et al., 2011; Borroto-Páez and Mancina, 2017; Rojas-Sandoval et al., 2017; Shiels et al., 2020). Islands within this region have been exposed to pronounced anthropogenic disturbance, which has included ecosystem degradation and natural resource overexploitation, particularly since the beginning of the European colonization (Maunder et al., 2008; Acevedo-Rodríguez and Strong, 2008). These disturbances often facilitated the introduction and establishment of non-native and invasive species (Lugo, 2004; Rojas-Sandoval and Acevedo-Rodríguez, 2015; Rojas-Sandoval et al., 2017, 2020). On the other hand, biodiversity on Caribbean islands is so remarkable that it is considered one of the most important hotspots of diversity in the world (Mittermeier et al., 2004). Elton's biotic resistance hypothesis proposes that areas of high diversity should be resistant to biological invasions (Elton, 1958). While this may be true at small scales, it is difficult to find evidence for it in larger regional scales (Stohlgren et al., 2003, 2006; Ackerman et al., 2017). Ackerman et al. (2017) evaluated Elton's theory at different scales using a dataset of plant species in Puerto Rico. They found that there was a strong, positive correlation between invasive and native species richness at large scales (municipality scale), yet the relationship between native and invasive species was weak at local forest scales (forest reserves), suggesting some biotic resistance is present at local forest reserves in Puerto Rico.

The island of Puerto Rico is the smallest of the Greater Antilles, and at the whole-island scale, these dual processes of biotic resistance vs. direct or indirect anthropogenic facilitation of species introductions are present. Occupied first by the indigenous peoples beginning 4,000 years BP, then colonized by the Spanish and eventually annexed by the United States, 95% of the island of Puerto Rico was converted to agriculture, mining, and urban areas (Birdsey and Weaver, 1987). Human activities were absent at the summits of the Luquillo Mountains, and these mountains have remained as the largest forested area on the island (**Figure 1**). Located in the Luquillo Mountains, El Yunque National Forest (EYNF) is currently the largest and the most well-conserved forest area in Puerto Rico. The lower flanks are a mosaic of former human land use, mostly farming and forestry, where forest has recovered naturally or has been partly restored by humans. The summits and higher elevation areas are relatively well-conserved remnants of native forest, away from roads that extend to two of the three dominant peaks.

In this study, we asked the following question: What is the current status of introduced species within El Yunque National Forest (EYNF), the largest and most well-conserved forest area of Puerto Rico? We intended to answer this question by first reviewing the literature on non-native species of vascular plants, invertebrates, and vertebrates that have been deliberately and/or accidentally introduced in EYNF, and then asking local experts to review the species list and identify and provide justification for those species that they considered to be currently behaving as invasive in EYNF. Based on previous studies showing that areas with higher anthropogenic disturbance often provide more opportunities for the introduction and establishment of non-native and invasive species (Lockwood et al., 2009; Blackburn et al., 2016; Dawson et al., 2017; Rojas-Sandoval et al., 2020), we hypothesized that areas within the EYNF with long-standing continuous native forest would have fewer non-native and invasive species than areas subject to high levels of anthropogenic disturbance such as those in the lowlands and areas near roads and other historical land-use sites.

METHODS

For the purposes of this review, we used the term "introduced" to signify non-native species brought to Puerto Rico intentionally or accidentally via human activities and have already established wild populations. We reserved "invasive" for non-native species that have been shown to spread rapidly and widely and are suspected or shown to have negative ecological or economic effects in the EYNF (Blackburn et al., 2011). Data presented here are based on published literature, museum and herbarium collections, first-hand experience from local and academic experts, and personal field observations. Using all this information, we first compiled a list of all the introduced species occurring within the EYNF including vertebrates, invertebrates, and vascular plants. Then, we identified invasive and potentially invasive species based on the abundance and the risk of causing negative impact on native communities within the EYNF. We found that some groups are better studied (e.g., vascular plants,

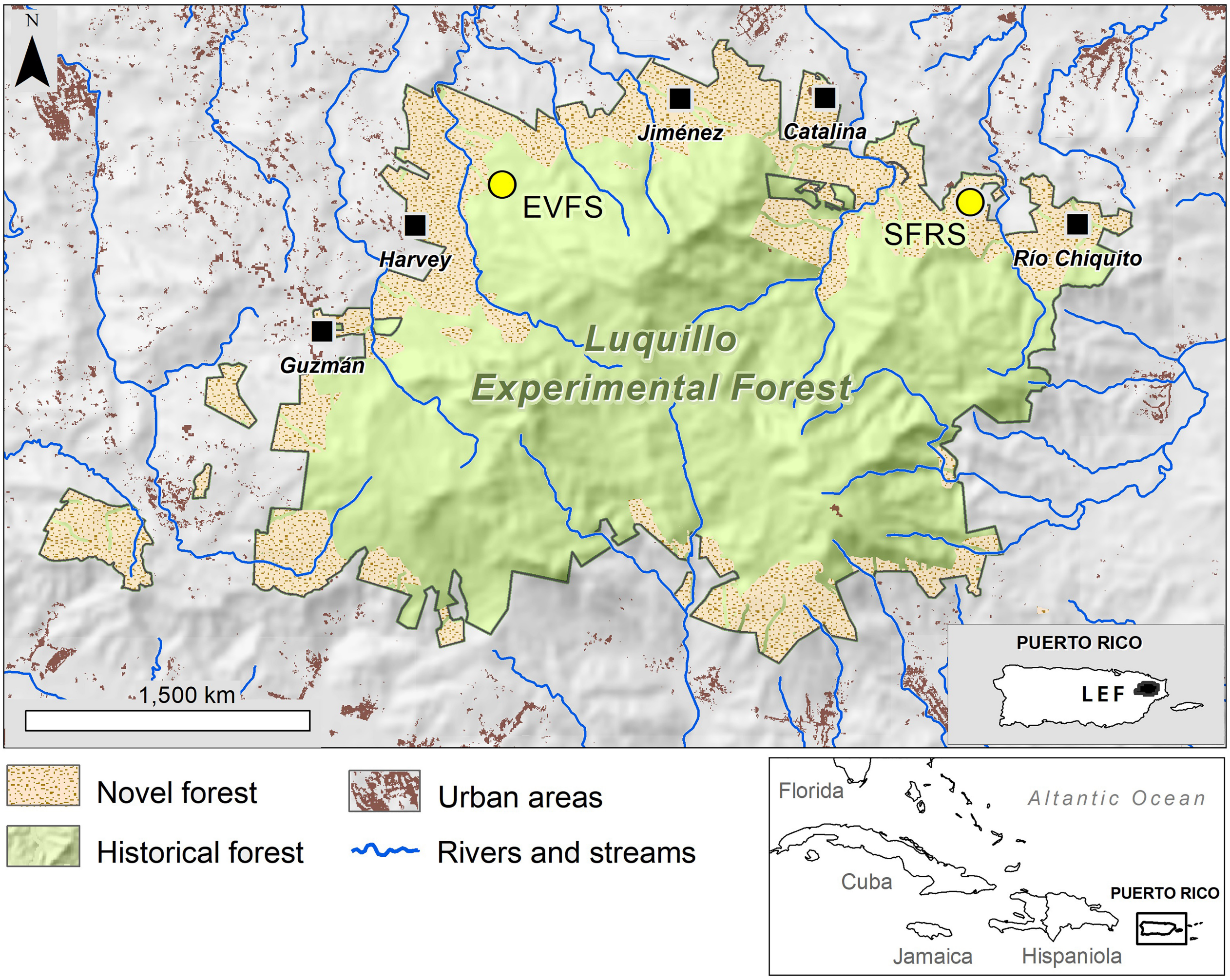

FIGURE 1 | Map of the Luquillo Experimental Forest (El Yunque National Forest) showing the distribution of historical forest (well-conserved forest with relatively little history of human disturbance) and novel forests (secondary forest and former plantations). Major study areas include El Verde Field Station (EVFS) and Sabana Field Research Station (SFRS); filled squares mark locations of plantations.

earthworms, and vertebrates) than others (e.g., arthropods) and few species have been thoroughly evaluated for their invasiveness (i.e., validated ecological or economic harm). Thus, our conclusions are preliminary in part and, where information is lacking, serve as a guide for future research.

RESULTS

Vascular Plants

In the EYNF, we identified 168 non-native plant species from 135 genera and 57 families indicating a very heterogeneous group in terms of their taxonomy, life forms, and ecological attributes (**Supplementary Table S1**). About 46% (77 species from the 168 non-native species) are species previously listed as invasive in Puerto Rico (O'Connor et al., 2000; Kairo et al., 2003; Brown et al., 2006; Cohen and Ackerman, 2009; Más and Lugo-Torres, 2013; Ackerman et al., 2014; Rojas-Sandoval and Acevedo-Rodríguez, 2015; Burman et al., 2017). When considering their invasive status within the EYNF, we identified a total of 37 species (**Table 1**). Most invaders in the EYNF are vines (14 species), herbs (9 species), and grasses (8 species) that are colonizing primarily disturbed areas (e.g., landslides), wastelands, river edges, and roadsides (**Figure 2** and **Table 1**). Only three species of non-native trees (*Schefflera actinophylla*, *Spathodea campanulate*, and *Syzygium jambos*) are regarded as invasive within the EYNF. There are just two plant species regarded as invasive in mature forest, and these included the tree *S. actinophylla* and the orchid *Oeceoclades maculata* (**Figure 2** and **Table 1**). We also found that many of the documented invaders in EYNF are species that were originally intentionally introduced into Puerto Rico as ornamentals (Valdés Pizzini et al., 2011; Areces-Berazain and Rojas-Sandoval, 2017) that have escaped cultivation (e.g., *Calathea lutea*, *Hedychium coronarium*, and *Spathoglottis plicata*). One example of this pathway of introduction is *Selaginella willdenowii*, a

TABLE 1 | Invasive plant species in El Yunque National Forest, Puerto Rico.

Species	Habitats invaded	Impacts/notes	References
Ardisia elliptica	Roadsides, disturbed areas along trails. Common by El Toro trail, from sea level to 600 + m. Rapidly spreading along Rd-186	Aggressive invader displacing and smothering native vegetation	J.D. Ackerman pers. comm.
Bambusa spp. (*B. vulgaris, B. longispiculata, B. tulda*, and *B. tuldoides*)	Disturbed areas, roadsides, riparian areas	Native vegetation, native stream macro-invertebrate communities	O'Connor et al., 2000; Blundell et al., 2003
Calathea lutea	Roadsides, disturbed areas. Rapidly spreading along Rd-191 and Portal-Sabana Rd	Native vegetation	Rojas-Sandoval, pers. obs.
Cenchrus purpureus	Roadsides, abandoned agricultural fields, lowlands, forest margins, disturbed areas	Native vegetation. Forming dense stands	Olander et al., 1998; Areces-Berazain et al., 2014
Coix lacryma-jobi	Riparian areas	Native riparian vegetation	Areces-Berazain and Rojas-Sandoval, 2017
Dendrocalamus strictus	Disturbed areas, roadsides, riparian areas	Native vegetation, native stream macro-invertebrate communities	O'Connor et al., 2000; Blundell et al., 2003
Dioscorea alata	Disturbed areas, forest margins, secondary forests at lower and middle elevations	Native vegetation	Acevedo-Rodríguez, 2005; EYNF, 2008
Epipremnum pinnatum	Disturbed areas, roadsides, secondary forests climbing on the trunks of trees and getting into the canopy	Native vegetation. Smothering native epiphytes	Acevedo-Rodríguez, 2005; Rojas-Sandoval and Acevedo-Rodríguez, 2015
Hedychium coronanium	Edges of shaded secondary forests, and disturbed areas along trails	Native vegetation. Forming dense stands	EYNF, 2008
Impatiens walleriana	Shaded secondary forest, roadsides	Native vegetation	Torres, 1992
Ipomoea spp. (*I. batatas, I. carnea, I. nil, I. ochracea, I. purpurea, I. quamoclit*, and *I. tricolor*)	Roadsides, waste places, thickets in secondary forests, forest margins, riparian areas	Locally abundant especially after hurricanes. Spreading and smothering native vegetation	Acevedo-Rodríguez, 2005; Ackerman, pers. comm.
Megathyrsus maximus	Roadsides, abandoned agricultural fields, lowlands, forest margins, disturbed areas	Native vegetation. Forming dense stands	Rojas-Sandoval and Acevedo-Rodríguez, 2015
Melinis minutiflora	Roadsides	Native vegetation	Olander et al., 1998
Nephrolepis brownii	Disturbed areas, roadsides, landslides	Native vegetation	Sharpe and Shiels, 2014; Shiels, pers. obs.
Oeceoclades maculata	Penetrates mature forest but is most abundant in areas with moderate levels of past disturbance	Native vegetation	Cohen and Ackerman, 2009
Paspalum fasciculatum	Roadsides, abandoned agricultural fields, lowlands, forest margins, disturbed areas	Native vegetation. Forming dense stands	Areces-Berazain et al., 2014
Pueraria phaseoloides	Disturbed areas, roadsides, forest margins, secondary forests	Spreading rapidly, forming dense colonies that engulf native vegetation	Acevedo-Rodríguez, 2005; EYNF, 2008
Schefflera actinophylla	Disturbed areas, secondary forests, and lowlands. Also invading primary forests	Native vegetation	Rojas-Sandoval and Acevedo-Rodríguez, 2015
Selaginella willdenowii	Roadsides. Very abundant along Rd-186	Spreading and smothering native vegetation	EYNF, 2008; Zimmerman, pers. obs.
Spathoglottis plicata	Roadsides, landslides, disturbed areas	Native vegetation, fungi	Ackerman et al., 2014
Spathodea campanulata	Lowlands, secondary forest	Native vegetation	Thompson et al., 2007
Sphagneticola trilobata	Roadsides, waste areas, landslides	Native vegetation	Shiels, pers. obs.
Syngonium podophyllum	Disturbed areas, roadsides, secondary forests	Form dense colonies that engulf native vegetation	Acevedo-Rodríguez, 2005; Rojas-Sandoval and Acevedo-Rodríguez, 2015
Syzygium jambos	Riparian areas, secondary forests, lowlands	Native vegetation	Brown et al., 2006; Burman et al., 2017
Thunbergia alata and *T. fragrans*	Roadsides, waste places, secondary forests, forest margins, riparian areas	Spreading rapidly, forming dense colonies that engulf native vegetation and shade-out native vegetation in the understory	Acevedo-Rodríguez, 2005; Rojas-Sandoval and Acevedo-Rodríguez, 2015
Talipariti tiliaceum	Roadsides, disturbed areas along trails. Dense thickets near El Toro trail	Native vegetation. Forming dense stands	EYNF, 2008
Tradescantia spathacea	Disturbed areas and roads along the wilderness boundary	Native vegetation	EYNF, 2008

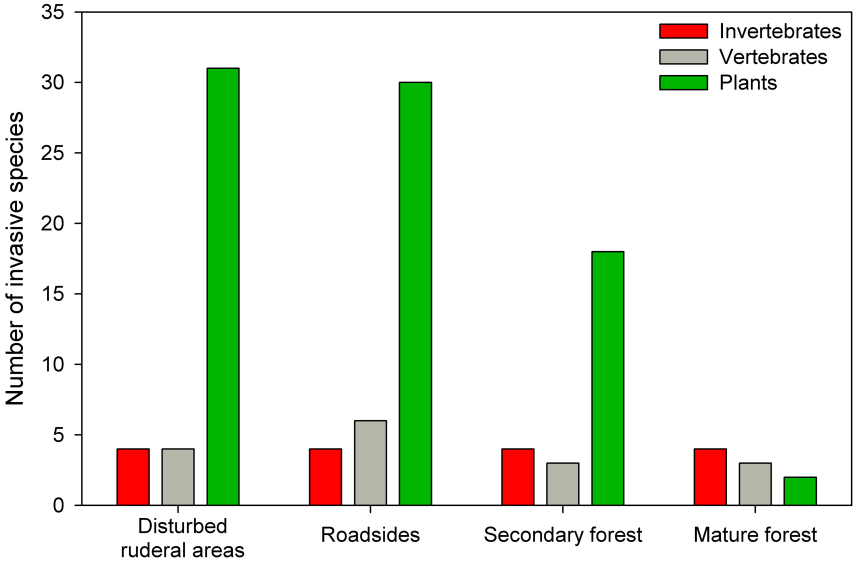

FIGURE 2 | Number of invasive invertebrate (n = 4), vertebrate (n = 6), and plant (n = 37) species and the different habitat types that they are invading within El Yunque.

TABLE 2 | Top invasive animal species in El Yunque National Forest (EYNF), Puerto Rico.

Species	Common name	Habitats invaded	Impact	References
Terrestrial vertebrates				
Felis catus	House cat	All	Birds, reptiles, invertebrates	Engeman et al., 2006
Herpestes auropunctatus	Mongoose	All	Birds, reptiles, invertebrates	Engeman et al., 2006
Rattus	Black rat	All	Native vegetation birds, reptiles, invertebrates	Shiels et al., 2018, 2019
Mus musculus	House mouse	Roadsides, grassy areas	Native vegetation invertebrates	Shiels et al., 2018
Rhinella marina	Cane toad	Roadsides	Reptiles, invertebrates	Stewart, 1995
Iguana	Green iguana	Roadsides	Native vegetation	Lugo, 2005
Terrestrial invertebrates				
Pontoscolex corethrurus	Amazonian earthworm	All	Invertebrate soil communities, soil dynamics, native vegetation	González et al., 2007
Ocnerodrilus occidentalis	Earthworm	All except Palo Colorado forest type	Invertebrate soil communities, soil dynamics, native vegetation	González et al., 2007
Apis mellifera hybrid	Africanized honeybee	All	Native parrots and other cavity nesting birds, humans	Blundell et al., 2003
Aedes aegypti	Yellow fever mosquito	All	Humans	Weinbren and Weinbren, 1970

popular ornamental spikemoss that escaped cultivation and has become one of the most aggressive plant invaders in EYNF (EYNF, 2008), and it can be found forming dense patches along forest margins near El Verde Field Station and elsewhere (**Figure 3**).

Our survey also revealed a prevalence of invasive vines (14 species) in the EYNF. Seven of the 14 species are morning-glory (*Ipomoea* spp.) that quickly respond to disturbances and have become a serious problem especially after hurricanes (**Table 1**). They display rapid growth and an ability to outcompete and smother native vegetation (J.D. Ackerman, personal communication). Moreover, vines such as *Epipremnum pinnatum*, *Pueraria phaseoloides*, *Dioscorea alata*, *Thunbergia alata*, and *Thunbergia fragrans* are also locally abundant across the EYNF and often can be found along roadsides colonizing forest margins.

In the case of grasses, three species were identified as the dominant invaders in the EYNF: *Cenchrus purpureus*, *Megathyrsus maximus*, and *Paspalum fasciculatum*. These three invaders are robust perennial C_4 grasses that grow rapidly, colonizing new areas and forming dense monospecific stands that displace native plants and wildlife. Across the EYNF, these grasses are locally abundant in disturbed areas and along roadsides mainly at low and middle elevation, but *C. purpureus* has also been reported invading disturbed areas at upper elevations (Olander et al., 1998).

Comprehensive studies of the distribution of native vs. non-native plants for the EYNF are missing. Rather, perspectives

FIGURE 3 | *Selaginella willdenowii*: one of the dominant invasive plant species that is rapidly spreading across El Yunque National Forest. This picture corresponds to a heavily invaded area along the PR-Highway 186. Photo: J. Zimmerman.

come from individual studies that focus on research areas or conspicuous species. For example, Thompson et al. (2007) found that 12 agricultural, mostly woody species (e.g., coffee, mango, and breadfruit) occurred within mature forest but exclusively in the northern two-thirds of the 16-ha Luquillo Forest Dynamics Plot. This area was traced to a history of logging and agriculture dating to the 1920s (Thompson et al., 2002). The remaining one-third of the plot located in the southern end had species representative of the native forest and no non-native species. Thus, the distribution of native and non-native plant species appears to be demarked by the "anthropogenic ecotone." None of these 12 introduced species were regarded as invasive by Thompson et al. (2007) since none of them were increasing strongly in abundance during the course of the 10-year study. One exception could be *Simarouba amara*, a tree species planted for timber across the Luquillo mountains, which has increased in numbers in areas of less intense land use since hurricane Georges passed through in 1998 (Thompson et al., 2007) and extensively since 2017 after hurricane Maria (Zimmerman, personal observation).

Brown et al. (2006) studied the ecology of *S. jambos* in the Luquillo Mountains with the goal of identifying whether this introduced ornamental was an invasive species. They concluded that where it occurs in areas of secondary forest within 30 m of stream beds, it is invasive and appears to outcompete other species due to its extreme shade tolerance. In their words: "After nearly 185 years since its introduction to the island, *S. jambos* is well-established" and "its presence does not appear limited," suggesting "a new vegetation assemblage in the regenerating secondary forests in the Luquillo Mountains." This case deserves further study as *S. jambos* is now the subject of an introduced pest, the guava rust, which appears to be negatively affecting its naturalized populations in Puerto Rico (Burman et al., 2017).

Another plant group that has been studied within the EYNF are bamboos. The planting of bamboo in this area started in the 1930s and has led to present-day bamboo monocultures in many riparian and roadside areas. Bamboos were originally introduced to stabilize recently constructed roadbeds (O'Connor et al., 2000; Blundell et al., 2003) and now there are about five species recognized as invasive in the EYNF (four species of *Bambusa*, and *Dendrocalamus strictus*; **Table 1**). Invasive bamboos are spreading along rivers and colonizing areas along roadsides, and they currently occupy about 2% of the forest area (O'Connor et al., 2000).

Terrestrial Invertebrates

In the EYNF, our survey for introduced arthropods was not as exhaustive as for plants, partly because of the bias of fewer studies and infrequent reporting of non-native arthropods. However, four species are regarded as invasive in this area: the Africanized honeybee (*Apis mellifera* of European origin hybridized with *A. mellifera scutella* of African origin; Rivera-Marchand et al., 2008), the mosquito, *Aedes aegypti*, and two species of introduced earthworms, *Pontoscolex corethrurus* and *Ocnerodrilus occidentalis* (González et al., 2007). We also found that these four invasive species are occurring ubiquitously across all habitat types in EYNF: from well-conserved mature forests to highly disturbed ruderal areas (**Figure 2** and **Table 2**).

In Puerto Rico, Africanized honeybee hybrids were first reported in 1994 (Cox, 1994), which was also the first report of these bee hybrids on an oceanic island. In the EYNF, Africanized honeybee hybrids have occupied 80% of tree cavities and threatened the endangered Puerto Rican Parrot (*Amazona vittata*) and other cavity nesting bird species (Blundell et al., 2003). Although it was several years ago, attacks to field researchers have been reported (Zimmerman, personal observation). As documented elsewhere on the island, the Africanized honeybees eventually evolved gentle behavioral characteristics and have ceased to be a great threat. The one persistent introduced insect in EYNF is the mosquito, *A. aegypti* (Weinbren and Weinbren, 1970). This mosquito, which originated in Africa and can be easily recognized by its white markings on the legs, is known as a vector of dengue fever, chikungunya, and Zika fever, and other human-disease agents. It is likely that *A. aegypti* is not reproducing in the elevations encompassed by EYNF, but instead the adult mosquitos observed within the EYNF could be transported by cars from San Juan and other low-elevation urban areas (Yee, pers. comm.).

González et al. (2007) studied the earthworm communities along the elevational gradient in the Luquillo Mountains, identifying eight different forest types from elfin forest at the summits to mangroves at the coast. Along this gradient, they found three introduced species of earthworms, *P. corethrurus*, *O. occidentalis*, and *Drawida barwelli*, and all of them are regarded as invasive species; however, *D. barwelli* was only found in lower-elevation forest areas located outside EYNF. *P. corethrurus*, a species introduced from Europe, was found in four forest types (i.e., elfin, palm, Colorado, and tabonuco) in the EYNF, and in one low-elevation forest type outside the EYNF. *O. occidentalis*, which is native to Central and South America (Shen et al., 2015), was found in all but one forest type (Colorado forest) in EYNF and in two additional lower-elevation forest types outside EYNF. These earthworm species are considered invasive because they alter biogeochemical cycling in the soil, which may further influence plant community dynamics (González et al., 2007). Additional invertebrates that may be of concern in the future, especially due to their ephemeral expansion after hurricanes, are non-native slugs and snails (e.g., *Allopeas gracile*; Bloch, unpubl. data). However, such invaders do not appear to be spreading rapidly or causing plant damage like they do on other islands such as Hawaii (e.g., Shiels et al., 2014).

Vertebrates

Our survey for introduced vertebrates identified a total of six species in the EYNF (**Table 2**), and all of them are regarded as invasive despite only some species occurring across all habitat types and others are confined to disturbed areas along roadsides and ruderal areas (**Figure 2** and **Table 2**). Out of the six, there are three dominant invasive mammals: black rats (*Rattus rattus*), mongoose (*Herpestes auropunctatus*), and feral cats (*Felis catus*). These three mammal species are widespread through disturbed and undisturbed mature forest (Engeman et al., 2006; Shiels et al., 2018), and each represents a threat to many native species, including the endangered Puerto Rican Parrot (*A. vittata*) (Engeman et al., 2006). In fact, some lethal trapping is regularly performed during the parrot nesting season to reduce local populations of these invasive mammals to mitigate their impact on the population of the endangered parrot (Engeman et al., 2006). Whereas rats and cats are acclaimed climbers, mongooses are not, but apparently, they consume birds that fall from the nest upon first flights (Engeman et al., 2006). Beyond parrots, diet studies of these three invasive mammals reveal their omnivorous behavior, as many species of native birds, reptiles, and invertebrates are susceptible to their consumption. Furthermore, a recent study of the black rat diet demonstrated that several native tree species (e.g., *Guarea guidonia*, *Buchenavia capitata*, and *Tetragastris balsamifera*) are at risk from seed removal and predation by rats in the EYNF (Shiels et al., 2019).

Another very common invasive species in EYNF is the house mouse (*Mus musculus*), which is a frequent invasive rodent of island ecosystems and it often coexists with invasive rats. While mice are typically less problematic for insular biodiversity than rats, their diet suggests that some native herbs and invertebrates are frequently consumed (Shiels et al., 2018). During a survey of small mammals along the elevation gradient of the PR-Highway 191 through EYNF, Shiels et al. (2018) found the presence of invasive back rats (*R. rattus*) at all elevations and habitat types, whereas house mice were also present at most elevations but were restricted to roadside habitats. Therefore, based on distribution and the range of dietary impact, it has been suggested that the black rat is a more damaging rodent in EYNF than the house mouse.

Two species of invasive herpetofauna have been reported in EYNF (**Table 2**), particularly near roadsides, and these include the cane toad (*Rhinella marina*) and green iguana (*Iguana iguana*). Whereas green iguanas are a relatively recent addition to the invasive fauna of EYNF (Lugo, 2005) and have been rarely observed in EYNF, cane toads have been established much longer and they are frequently seen nocturnally after heavy rains (Stewart, 1995).

For birds, Wunderle and Arendt (2011) provide a list of 99 bird species found in EYNF, including 23 species that breed there. Twelve breeding species are endemic. No introduced bird species are found in the EYNF, even in areas with a history of anthropogenic disturbance. Naturalized species of finches and psittacines are otherwise common on the forest's periphery, mostly in anthropogenic-disturbed habitats (Vázquez Plass and Wunderle, 2013). Similarly, no introduced species of fish are found in streams of the EYNF except anecdotal findings of aquarium species in recreation areas. These non-native species apparently do not survive frequent flood events (Ramirez, pers. comm.). Non-native fish species are found in urban streams in areas nearby the EYNF and increases in their abundances are facilitated by drought events (Ramírez et al., 2018). Domestic dogs (*Canis familiaris*) are occasionally abandoned along lowland roads of EYNF, but they rarely establish and are not spreading to our knowledge.

DISCUSSION

As the theory of island biogeography predicts, the island of Puerto Rico is relatively poor in species richness compared to mainland tropical areas, with concomitantly reduced biological complexity. For example, among comparable-sized areas (16–25 ha), in a forest dynamics plot (FDP) in Puerto Rico (in wet forest), there are as many as 150 freestanding woody species, while one plot in central Panama has ∼300 species and one in Ecuador has > 1,000 species (Condit et al., 2005; Ostertag et al., 2014). Similarly, the food web at El Verde, a field station located within the EYNF, shows a complete absence of large herbivores and predators, low faunal richness compared to the tropical mainland, and an abundance of frogs and lizards (Reagan and Waide, 1996).

Nevertheless, Puerto Rico is much more diverse than other more isolated islands from other regions. For example, the 4-ha FDP in Palamanui, Hawaii has only 15 tree species (Ostertag et al., 2014). Does this roughly 10-fold difference in plant species richness between EYNF and Palamanui impart any resistance to species introductions for EYNF? A widely recognized pattern in island biogeography is the species–isolation relationship (SIR), in which a decrease in the number of native species on oceanic islands will occur with increasing island isolation, linked to lower rates of natural dispersion and colonization on the remotest islands (Wilson and MacArthur, 1967). While the negative SIR has been well-documented for native species, the response of non-native species to geographic isolation is less clear and remains an open question. In this regard, Moser et al. (2018) tested the SIR for a large dataset of native and non-native species on islands worldwide and they found that the number of introduced species increased with island isolation for all taxa studied except for birds, which is a pattern that is opposite to the widely recognized negative SIR for native species.

Moser et al. (2018) argue that this pattern is due to reduced native diversity and greater ecological naiveté of native biota on more remote islands. Furthermore, their analyses removed the influence of factors such as island size, climatic and topographic heterogeneity, and socioeconomic development [using per capita gross domestic product (GDP) as a proxy]. Thus, the expectation is that, all else being equal, the close biogeographic history of Puerto Rico and nearby mainland areas should provide a relative degree of biotic resistance to invasions of new species into the island's biotic communities.

Among the important elements set aside by Moser et al. (2018) was anthropogenic disturbance, which has factored considerably in Puerto Rico, and the Caribbean in general (Kueffer et al., 2010; Rojas-Sandoval et al., 2017, 2020). Puerto Rico currently has one of the highest GDPs in the Caribbean and a high population density (Kueffer et al., 2010; Rojas-Sandoval et al., 2017) relative to many other islands, and its original forest cover was reduced to ∼5% (Birdsey and Weaver, 1987), all indicating a high degree of human disturbance. All of this has led to a high rate of introduction and naturalization of non-native and invasive species. For example, Rojas-Sandoval and Acevedo-Rodríguez (2015) found that about 32% of the total flora in Puerto Rico is introduced, a percentage relatively high when compared to other islands in the Greater Antilles such as Cuba (12%), Dominican Republic (18%), and Jamaica (21%). For Puerto Rico and other Caribbean islands, previous studies have shown that successful establishment of non-native plant species is more likely to occur in human-modified habitats than in pristine habitats. On these islands, disturbance and human-related activities seem to be major drivers influencing and facilitating the introduction and establishment of non-native plant species (Kueffer et al., 2010; Rojas-Sandoval and Acevedo-Rodríguez, 2015; Ackerman et al., 2017; Rojas-Sandoval et al., 2017, 2020).

To understand the diversity and richness of invasive species in EYNF, it is necessary to take into account the socioeconomic history of human-modified areas where farmland and logging occurred until the 1930s (e.g., Thompson et al., 2002). Our initial hypothesis was that undisturbed natural areas within EYNF would harbor fewer non-native species and be more resistant to invasion than disturbed areas away from protected areas. The information we garnered in our literature reviews and surveys of experts lends only partial support of this hypothesis. In general, vascular plants seem to hold to the pattern quite well, and a large number of the non-native plant species were more common in disturbed areas with a history of agriculture or other human land use. We found no evidence from managers suggesting that those species are rapidly spreading into better-conserved forest areas (Thompson et al., 2007; EYNF, 2008). On the other hand, the non-native vertebrates and invertebrates currently invading EYNF were not restricted in their distributions, for the most part. For example, introduced earthworms were found in both disturbed and undisturbed forests and at elevations covering all forest types described for EYNF. Similarly, invasive mammals such as black rats, cats, and mongoose roam freely throughout EYNF, and at least, the rats do not appear to be more active or abundant in disturbed habitats such as roads, landslides, riparian areas, or treefall and hurricane gaps relative to the interior mature forest (Shiels et al., 2018, 2019). Birds and fish were exceptions, with introduced species not found even in areas with long histories of human disturbance. One outcome that is clear from our review is the lack of detailed information on the distribution and abundance of non-native species within EYNF, as well as the concomitant lack of information on ecological or economic impacts of non-native species and their classification as invasive species. One of the caveats of this work is that the information that we were able to obtain was for the most part too coarse in both space and time to fully evaluate our hypothesis.

Few species have been fully studied in EYNF for their invasiveness as we have defined it. Six plant species, *S. jambos* and five bamboos (four *Bambusa* spp. and *D. strictus*), appear to ecologically dominate riparian areas where they were initially planted and are slowly spreading across the area (O'Connor et al., 2000; Brown et al., 2006). Additional plant species such as *S. wildenowii*, *Ipomoea* vines, and the grasses *C. purpureus*, *M. maximus*, and *P. fasciculatum* are behaving as invaders and forming dense patches that outcompete and smother native vegetation, and thus, they have been on the radar of managers (EYNF, 2008). Introduced earthworms are altering soil processes (González et al., 2007) and could potentially influence other ecological processes. Africanized honeybees and *A. aegypti* mosquitos threaten human visitors to EYNF. Introduced black rats are a nuisance and may cause significant harm to native vegetation (Shiels et al., 2019) and birds, while mongooses, rats, and feral cats may prey on critically endangered wildlife (Engeman et al., 2006). In sum, we find some worrisome trends in the effects of invasive species but no strong evidence that the EYNF ecosystem as a whole is challenged by these invaders such that a novel ecosystem will take over.

Our combined results showed that many non-native and invasive species in the EYNF are yet confined to the lowlands and to areas with high levels of anthropogenic disturbance. However, accurate data on their distribution, abundance, and impacts are very limited. Therefore, systematic surveys and detailed studies monitoring non-native and invasive species are needed to draw conclusions on the invasive potential and social, economic, and/or ecological impacts caused by non-native invasive species within this protected area. Considering that EYNF is one of the few (and the most important) remnants of original native forest in Puerto Rico, the potential of significant impacts by invasive species on its unique native biodiversity is high. The control and management of the current and potentially invasive species within EYNF should remain a high priority.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

JZ initiated the study, provided guidance, wrote much of an initial draft, and finalized the text. JR-S analyzed plant distribution data and contributed to synthesis and writing. AS synthesized information on animals and contributed to the writing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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REFERENCES

Acevedo-Rodríguez, P. (2005). Vines and climbing plants of Puerto Rico and the Virgin Islands. *Contrib. U S Natl. Herb.* 51:483.

Acevedo-Rodríguez, P., and Strong, M. T. (2008). Floristic richness and affinities in the West Indies. *Bot. Rev.* 74, 5–36. doi: 10.1007/s12229-008-9000-9001

Ackerman, J. D., Falcón, W., Molinari, J., Vega, C., Espino, I., and Cuevas, A. A. (2014). Biotic resistance and invasional meltdown: consequences of acquired interspecific interactions for an invasive orchid, Spathoglottis plicata in Puerto Rico. *Biol. Invasions* 16, 2435–2447. doi: 10.1007/s10530-014-0676-3

Ackerman, J. D., Tremblay, R. L., Rojas-Sandoval, J., and Hernández-Figueroa, E. (2017). Biotic resistance in the tropics: patterns of seed plant invasions within an island. *Biol. Invasions* 19, 315–328. doi: 10.1007/s10530-016-1281-4

Areces-Berazain, F., and Rojas-Sandoval, J. (2017). "Coix lacryma-jobi" in *Invasive Species Compendium*. Wallingford, UK: CAB International. Available online at: www.cabi.org/isc.

Areces-Berazain, F., Vega López, V. J., and Ackerman, J. D. (2014). "Annotated list of the vascular plants at el verde field station, el yunque national forest," *Puerto Rico.* Caribbean Naturalist, 1–51.

Birdsey, R. A., and Weaver, P. L. (1987). *Forest Area Trends in Puerto Rico. Res. Note SO-331*. New Orleans, LA: US Department of Agriculture, Forest Service, Southern Forest Experiment Station.

Blackburn, T. M., Delean, S., Pyšek, P., and Cassey, P. (2016). On the island biogeography of aliens: a global analysis of the richness of plant and bird species on oceanic islands. *Global Ecol. Biogeography* 25, 859–868. doi: 10.1111/geb.12339

Blackburn, T. M., Pyšek, P., Bacher, S., Carlton, J. T., Duncan, R. P., Jarošík, V., et al. (2011). A proposed unified framework for biological invasions. *Trends Ecol. Evol.* 26, 333–339. doi: 10.1016/j.tree.2011.03.023

Blundell, A. G., Scatena, F. N., Wentsel, R., and Sommers, W. (2003). Ecorisk assessment using indicators of sustainability: invasive species in the Caribbean National Forest of Puerto Rico. *J. Forestry* 101, 14–19.

Borroto-Páez, R., and Mancina, C. A. (2017). Biodiversity and conservation of Cuban mammals: past, present, and invasive species. *J. Mammal.* 98, 964–985. doi: 10.1093/jmammal/gyx017

Brown, K. A., Scatena, F. N., and Gurevitch, J. (2006). Effects of an invasive tree on community structure and diversity in a tropical forest in Puerto Rico. *Forest Ecol. Management* 226, 145–152. doi: 10.1016/j.foreco.2006.01.031

Burman, E., Ackerman, J. D., and Tremblay, R. L. (2017). Invasive Syzygium jambos trees in Puerto Rico: no refuge from guava rust. *J. Trop. Ecol.* 33:205. doi: 10.1017/s026646741700013x

Capinha, C., Essl, F., Seebens, H., Moser, D., and Pereira, H. M. (2015). The dispersal of alien species redefines biogeography in the Anthropocene. *Science* 348, 1248–1251. doi: 10.1126/science.aaa8913

Cohen, I. M., and Ackerman, J. D. (2009). *Oeceoclades maculata*, an alien tropical orchid in a Caribbean rainforest. *Ann. Bot.* 104, 557–563. doi: 10.1093/aob/mcn191

Condit, R. S., Ashton, M. S., Balslev, H., Brokaw, N. V., Bunyavejchewin, S., Chuyong, G. B., et al. (2005). Tropical tree a-diversity: results from a worldwide network of large plots. *Biologiske Skrifter* 55, 565–582.

Cox, B. (1994). AHB in Puerto Rico. *American Bee Journal* 134, 668–669.

Dawson, W., Moser, D., Van Kleunen, M., Kreft, H., Pergl, J., Pyšek, P., et al. (2017). Global hotspots and correlates of alien species richness across taxonomic groups. *Nat. Ecol. Evol.* 1:186.

Elton, C. S. (1958). *The Ecology of Invasions by Plants and Animals*. London: Methuen.

Engeman, R., Whisson, D., Quinn, J., Cano, F., Quiñones, P., and White, T. H. Jr. (2006). Monitoring invasive mammalian predator populations sharing habitat with critically endangered Puerto Rican parrot Amazona vittata. *Oryx* 40, 95–102. doi: 10.1017/s0030605305001286

EYNF (2008). *El Yunque National Forest Fiscal Year 2008 Monitoring and Evaluation Report*. https://www.fs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb5304902.pdf (accessed May 6, 2020)

Gallardo, B., Zieritz, A., and Aldridge, D. C. (2015). The importance of the human footprint in shaping the global distribution of terrestrial, freshwater and marine invaders. *PLoS One* 10:e0125801. doi: 10.1371/journal.pone.0125801

González, G., García, E., Cruz, V., Borges, S., Zalamea, M., and Rivera, M. M. (2007). Earthworm communities along an elevational gradient in northeastern Puerto Rico. *Eur. J. Soil Biol.* 43, S24–S32.

Kairo, M., Ali, B., Cheesman, O., Haysom, K., and Murphy, S. (2003). *Invasive Species Threats in the Caribbean Region*. Arlington, TX: Report to the Nature Conservancy.

Kueffer, C., Daehler, C. C., Torres-Santana, C. W., Lavergne, C., Meyer, J. Y., Otto, R., et al. (2010). A global comparison of plant invasions on oceanic islands. *Perspect. Plant Ecol. Evol. Syst.* 12, 145–161. doi: 10.1016/j.ppees.2009.06.002

Lockwood, J. L., Cassey, P., and Blackburn, T. M. (2009). The more you introduce the more you get: the role of colonization pressure and propagule pressure in invasion ecology. *Divers. Distrib.* 15, 904–910. doi: 10.1111/j.1472-4642.2009.00594.x

Lugo, A. E. (2004). The outcome of alien tree invasions in Puerto Rico. *Front. Ecol. Environ.* 5:265–273. doi: 10.2307/3868267

Lugo, A. E. (2005). The ecosystems of the Luquillo Mountains. *Acta Sci.* 19, 3–6.

Más, E. G., and Lugo-Torres, M. D. L. (2013). *Common Weeds in Puerto Rico and US Virgin Islands*. Puerto Rico: University of Puerto Rico and the USDA Natural Resources Conservation Service.

Maunder, M., Leiva, A., and Santiago-Valentin, E. (2008). Plant conservation in the Caribbean island biodiversity hotspot. *Bot. Rev.* 74, 197–207. doi: 10.1007/s12229-008-9007-7

Mittermeier, R. A., Gil, R. R., and Hoffman, M. (2004). *Hotspots Revisited: Earth's Biologically Richest and Most Threatened Terrestrial Ecoregions*. Mexico, DF: CEMEX.

Moser, D., Lenzner, B., Weigelt, P., Dawson, W., Kreft, H., Pergl, J., et al. (2018). Remoteness promotes biological invasions on islands worldwide. *Proc. Natl. Acad. Sci. U S A.* 115, 9270–9275. doi: 10.1073/pnas.1804179115

O'Connor, P. J., Covich, A. P., Scatena, F. N., and Loope, L. L. (2000). Non-indigenous bamboo along headwater streams of the Luquillo Mountains, Puerto Rico: leaf fall, aquatic leaf decay and patterns of invasion. *J. Trop. Ecol.* 16, 499–516. doi: 10.1017/s0266467400001541

Olander, L. P., Scatena, F. N., and Silver, W. L. (1998). Impacts of disturbance initiated by road construction in a subtropical cloud forest in the Luquillo Experimental Forest, Puerto Rico. *Forest Ecol. Management* 109, 33–49. doi: 10.1016/s0378-1127(98)00261-8

Ostertag, R., Inman-Narahari, F., Cordell, S., Giardina, C. P., and Sack, L. (2014). Forest structure in low-diversity tropical forests. *PLoS One* 9:e103268. doi: 10.1371/journal.pone.0103268

Ramírez, A., Gutiérrez-Fonseca, P. E., Kelly, S. P., Engman, A. C., Wagner, K., Rosas, K. G., et al. (2018). Drought facilitates species invasions in an urban stream: results from a long-term study of tropical island fish assemblage structure. *Front. Ecol. Evol.* 6:115. doi: 10.3389/fevo.2018.00115

Reagan, D. P., and Waide, R. B. (eds) (1996). *The Food Web of a Tropical Rain Forest*. Chicago, IL: University of Chicago Press.

Reynolds, R. G., and Niemiller, M. L. (2010). Island invaders: introduced amphibians and reptiles in the Turks and Caicos Islands. *Reptiles Amphibians* 17, 117–121.

Rivera-Marchand, B., Giray, T., and Guzmán-Novoa, E. (2008). The cost of defense in social insects: insights from the honeybee. Entomol. Experiment. Appl. 129, 1–10. doi: 10.1111/j.1570-7458.2008.00747.x

Rojas-Sandoval, J., and Acevedo-Rodríguez, P. (2015). Naturalization and invasion of alien plants in Puerto Rico and the Virgin Islands. *Biol. Invasions* 17, 149–163. doi: 10.1007/s10530-014-0712-3

Rojas-Sandoval, J., Ackerman, J. D., and Tremblay, R. L. (2020). Island biogeography of native and alien plant species: contrasting drivers of diversity across the Lesser Antilles. *Diversity Distrib.* 26, 1539–1550. doi: 10.1111/ddi.13139

Rojas-Sandoval, J., Tremblay, R. L., Acevedo-Rodríguez, P., and Díaz-Soltero, H. (2017). Invasive plant species in the West Indies: geographical, ecological, and floristic insights. *Ecol. Evol.* 7, 4522–4533. doi: 10.1002/ece3.2984

Roncal, J., Nieto-Blázquez, M. E., Cardona, A., and Bacon, C. D. (2020). "Historical Biogeography of Caribbean Plants Revises Regional Paleogeography," in *Neotropical Diversification: Patterns and Processes*, (Berlin: Springer), 521–546. doi: 10.1007/978-3-030-31167-4_20

Sharpe, J. M., and Shiels, A. B. (2014). Understory fern community structure, growth and spore production responses to a large-scale hurricane experiment in a Puerto Rico rainforest. *Forest Ecol. Manag.* 332, 75–86. doi: 10.1016/j.foreco.2014.01.023

Shen, H. P., Chang, C. H., and Chih, W. J. (2015). Earthworms from Matsu, Taiwan with descriptions of new species of the genera Amynthas (Oligochaeta: Megascolecidae) and Drawida (Oligochaeta: Moniligastridae). *Zootaxa* 3973:425. doi: 10.11646/zootaxa.3973.3.2

Shiels, A. B., Ennis, M. K., and Shiels, L. (2014). Trait-based plant mortality and preference for native vs. non-native seedlings by invasive slug and snail herbivores in Hawaii. *Biol. Invasions* 16, 1929–1940. doi: 10.1007/s10530-013-0636-3

Shiels, A. B., Lombard, C. D., Shiels, L., and Hillis-Starr, Z. (2020). Invasive rat establishment and changes in small mammal populations on Caribbean islands following two hurricanes. *Global Ecol. Conserv.* 22:e00986. doi: 10.1016/j.gecco.2020.e00986

Shiels, A. B., Ramírez, and de Arellano, G. E. (2018). Invasive rats (Rattus sp.), but not always mice (Mus musculus), are ubiquitous at all elevations and habitats within the Caribbean National Forest, Puerto Rico. *Caribbean Nat.* 48, 1–14.

Shiels, A. B., Ramírez, and de Arellano, G. E. (2019). Habitat use and seed removal by invasive rats (Rattus rattus) in disturbed and undisturbed rainforest, Puerto Rico. *Biotropica* 51, 378–386. doi: 10.1111/btp.12640

Stewart, M. M. (1995). Climate driven population fluctuations in rain forest frogs. *J. Herpetol.* 29, 437–446. doi: 10.2307/1564995

Stohlgren, T. J., Barnett, D. T., and Kartesz, J. T. (2003). The rich get richer: patterns of plant invasions in the United States. *Front. Ecol. Environ.* 1:11–14. doi: 10.2307/3867959

Stohlgren, T. J., Jarnevich, C., Chong, G. W., and Evangelista, P. H. (2006). Scale and plant invasions: a theory of biotic acceptance. *Preslia* 78, 405–426.

Thompson, J., Brokaw, N., Zimmerman, J. K., Waide, R. B., Everham, E. M. I. I. I., Jean Lodge, D., et al. (2002). Land use history, environment, and tree composition in a tropical forest. *Ecol. Appl.* 12, 1344–1363. doi: 10.1890/1051-0761(2002)012[1344:luheat]2.0.co;2

Thompson, J., Lugo, A. E., and Thomlinson, J. (2007). Land use history, hurricane disturbance, and the fate of introduced species in a subtropical wet forest in Puerto Rico. *Plant Ecol.* 192, 289–301. doi: 10.1007/s11258-007-9318-5

Torres, J. A. (1992). Lepidoptera outbreaks in response to successional changes after the passage of hurricane hugo in puerto rico. *J. Trop. Ecol.* 8, 285–298.

Valdés Pizzini, M., González Cruz, M., and Martínez Reyes, J. E. (2011). *La Transformación del Paisaje Puertorriqueño y la Disciplina del Cuerpo Civil de Conservación, 1933–1942*. San Juan: CIS-Universidad de Puerto Rico.

Vázquez Plass, E. O., and Wunderle, J. M. Jr. (2013). Avian distribution along a gradient of urbanization in northeastern Puerto Rico. *Ecol. Bull.* 54, 141–156.

Weinbren, M. P., and Weinbren, B. M. (1970). "Observations on the mosquito population in the irradiated forest at El Verde," in *A Tropical Rain Forest: a Study of Irradiation and Ecology at El Verde, Puerto Rico*, eds H. T. Odum and R. F. Pigeon (Springfield, VA: U.S. Atomic Energy Commission Division of Technical Information, National Technical Information Service), E159—-E167.

Wilson, B. S., Koenig, S. E., van Veen, R., Miersma, E., and Rudolph, D. C. (2011). Cane toads a threat to West Indian wildlife: mortality of Jamaican boas attributable to toad ingestion. *Biol. Invasions* 13, 55–60. doi: 10.1007/s10530-010-9787-7

Wilson, E. O., and MacArthur, R. H. (1967). *The Theory of Island Biogeography*. Princeton, NJ: Princeton University Press.

Wunderle, J. M., and Arendt, W. J. (2011). Avian studies and research opportunities in the Luquillo Experimental Forest: a tropical rain forest in Puerto Rico. *Forest Ecol. Manag.* 262, 33–48. doi: 10.1016/j.foreco.2010.07.035

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Honey Bee Diversity Is Swayed by Migratory Beekeeping and Trade Despite Conservation Practices: Genetic Evidence for the Impact of Anthropogenic Factors on Population Structure

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The intense admixture of honey bee (*Apis mellifera* L.) populations at a global scale is mostly attributed to the widespread migratory beekeeping practices and replacement of queens and colonies with non-native races or hybrids of different subspecies. These practices are also common in Anatolia and Thrace, but their influence on the genetic make-up of the five native subspecies of honey bees has not been explored. Here, we present an analysis of 30 microsatellite markers from honey bees from six different regions in Anatolia and Thrace ($N = 250$ samples), with the aim of comparing the impact of: (1) migratory beekeeping, (2) queen and colony trade, and (3) conservation efforts on the genetic structure of native populations. Populations exposed to migratory beekeeping showed less allegiance than stationary ones. We found genetic evidence for migratory colonies, acting as a hybrid zone mobile in space and time, becoming vectors of otherwise local gene combinations. The effect of honey bee trade leaves very high introgression levels in native honey bees. Despite their narrow geographic range, introgression occurs mainly with the highly commercial Caucasian bees. We also measured the direction and magnitude of gene flow associated with bee trade. A comparison between regions that are open and those closed to migratory beekeeping allowed the evaluation of conservation sites as centers with limited gene flow and demonstrated the importance of establishing such isolated regions. Despite evidence of gene flow, our findings confirm high levels of geographically structured genetic diversity in four subspecies of honey bees in Turkey and emphasize the need to develop policies to maintain this diversity. Our overall results are of interest to the wider scientific community studying anthropogenic effects on the population diversity of honey bees and other insects. Our findings on the effects of migratory beekeeping, replacement of queens and colonies have implications for the conservation of honey bees, other pollinators, and invertebrates, in general, and are informative for policy-makers and other stakeholders in Europe and beyond.

Keywords: queen and colony trade, gene flow, population structure, biodiversity conservation, microsatellite markers, *Apis mellifera* subspecies, isolated regions, migratory beekeeping

INTRODUCTION

The Western honey bee, *Apis mellifera* L., plays an important role, together with other pollinators, in the pollination of wild and cultivated plants. Likewise, honey bees have significant economic importance due to their production of honey and other products (Morse, 1991; Breeze et al., 2011). In addition to their ecological and economic importance, honey bees serve as model organisms for the study of fundamental questions on sociality and on cognition (Weinstock et al., 2006).

The natural distribution of *A. mellifera* includes Central and Southwest Asia, Europe, and Africa but the species was also introduced to East and Southeast Asia, Australia, and the Americas, mainly for its economic benefits (Ruttner, 1988). Morphological and molecular studies point to four major lineages of numerous—more than 20—subspecies (Ruttner, 1988; Whitfield et al., 2006). The four widely recognized lineages are A (Africa), M (western and northern Europe), O (Near East and Central Asia), and C (Eastern Europe) lineages.

In the past decade, various molecular-based studies have garnered support for the hypothesis that *A. mellifera* originated in the African tropics or subtropics and colonized its present European range by two main routes: through the strait of Gibraltar and through the Suez and then Bosporus regions, with a subsequent contact between the highly divergent M and C lineages in the region surrounding the Alps (Whitfield et al., 2006; Han et al., 2012; Harpur et al., 2014; Wallberg et al., 2014; Cridland et al., 2017).

Honey bees and wild pollinators are experiencing a worldwide decline due to factors closely related to human activities. Levels of decline vary and are related to species and geographic region. Some of the anthropogenic factors implicated in the decline are the destruction and fragmentation of natural habitats, toxicity caused by pollution and pesticides—such as the widely used neonicotinoids—and diseases. The latter is being facilitated by the spread of invasive species (Meffe, 1998; Brown and Paxton, 2009; Van Engelsdorp and Meixner, 2010; Blacquiere et al., 2012). Wild populations of honey bee species including feral populations in the genus *Apis* also have been negatively affected, namely, *Apis cerana*, *Apis florea*, *Apis dorsata*, and other native bees of Asia (Oldroyd, 2007; Dietemann et al., 2009; Van Engelsdorp et al., 2009; Genersch, 2010; Evans and Schwarz, 2011).

In addition, native honey bees are experiencing local losses, extinction, and/or genetic swamping as a result of genetic admixture due to bee trade, including the replacement of local bees with non-native strains and the beekeeping practice of moving colonies between geographic areas (De la Rúa et al., 2009).

The above genetic and environmental factors, and their interaction, have a cumulative adverse effect on honey bees and likely contribute to continuous or discrete events of sudden colony losses. This Colony Collapse Disorder (CCD) or Colony Depopulation Syndrome (CDS) (Van Engelsdorp et al., 2009; Neumann and Carreck, 2010) as it is referred to, is characterized by rapid depletion of worker bees while the queen continues egg laying and is accompanied by a lack of dead bees in and around the hive.

Honey bees may be able to adapt to this new challenge by relying on the adaptations and genetic diversity they accumulated over their evolutionary history. Honey bee subspecies perform differently in different environments and some locally adapted populations may display greater resistance to anthropogenic threats (Büchler et al., 2014). Hence, research on honey bee genetic diversity at the individual, colony, population, ecotype, and subspecies level is of great importance for safeguarding the species, the ecosystem, and the economic services they provide.

Recent research conducted on European honey bee population structure has shown that past distribution patterns have been disturbed (Dall'Olio et al., 2007; Bouga et al., 2011; Cánovas et al., 2011). In Africa, introgression of non-native DNA was detected in wild populations of Sudan (El-Niweiri and Moritz, 2010). The causes for these disturbances have been attributed mainly to queen and colony trade, replacement of native honey bees with non-natives as well as migratory beekeeping. However, there are very few studies on the direct genetic consequences of human practices on honey bee diversity.

Turkey has five subspecies of honey bees within its borders and beekeepers practice a variety of strategies, thus it provides an ideal environment to test the impact of anthropogenic factors on one of the most important pollinators of crops and wild plants.

Beekeeping in the region of Anatolia is a practice dating back to 6600 BC when the Hittite civilization presided over this region (Akkaya and Alkan, 2007). Beekeeping has been persevered and continues to be intensively practiced in Turkey where there are more than eight million hives distributed throughout the country. This is the third-highest number of hives in a single country. It is three times higher than the number of hives in the United States and reaches half of the EU countries total (European Parliament, 2017; USDA NASS, 2019).

As a reflection of the long association of the genus *Apis* with the region of Anatolia, one-fourth to one-fifth of the recognized subspecies of *A. mellifera*, namely, *A. m. meda*, *A. m. syriaca*, *A. m. caucasica*, and *A. m. anatoliaca* from the O-lineage and an ecotype from the C subspecies group occur in Turkey (Kandemir et al., 2005). In addition, A-lineage genetic material was also detected in native bees from the Levantine coast of Turkey (Kandemir et al., 2006) bringing together genetic elements from three continents, Africa, Europe, and Asia. The major subspecies found in and around Anatolia are shown in **Figure 1A**.

Together, Anatolia and Thrace harbor a vast diversity of honey bees belonging to three different lineages. In this region, they meet, exchange genes, and adapt to local conditions determined by local diverse climatic, topographical, and floristic variations (Bouga et al., 2011). The refugial status of Anatolia during the ice ages contributed to the enhanced levels of the present floral and faunal biodiversity (Hewitt, 1999). Studies of Turkish honey bee populations (Bodur et al., 2007; Kence et al., 2009) demonstrated high genetic structuring and confirmed the presence of divergent populations pointing to different subspecies. These researchers pointed to the rich diversity of honey bee populations in Anatolia and Thrace, and highlighted that they are under threat and that there is an urgent need to take steps for their conservation.

However, despite the above research, arguments prevail in the beekeeping environment that locally adapted honey bee ecotypes

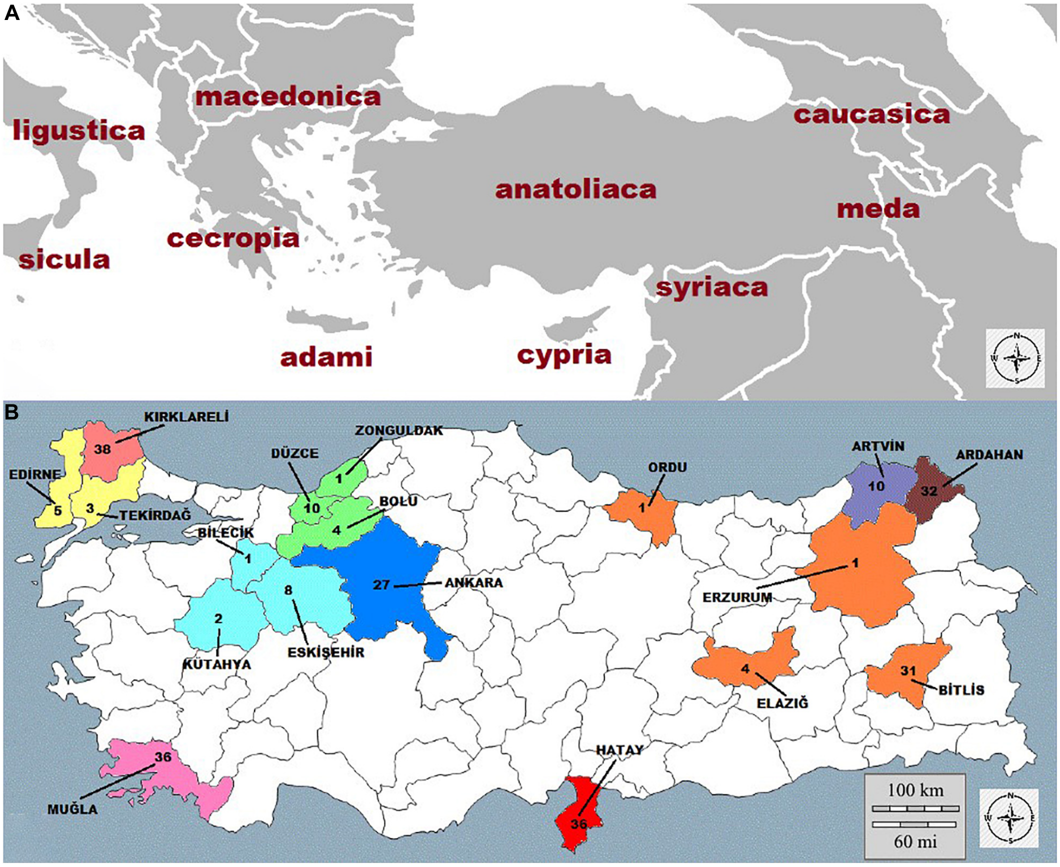

FIGURE 1 | Geographic distribution of **(A)** major honey bee (*A. mellifera*) subspecies in and around Anatolia **(B)** sampling sites and sample sizes.

have been irrevocably lost, due to gene flow, and thus steps to safeguard locally adapted honey bee variants do not have merit. This argument is further strengthened since queen bee trade is not currently subject to any restrictions or regulations in Turkey. There are few pioneering measures of conservation within the natural distribution range of the subspecies, likely not enough to guarantee the preservation of genetic structure in the next decades.

The aim of this study is to investigate the impact of anthropogenic factors and conservation efforts on the current pattern of genetic diversity of honey bee populations in Turkey. Should genetic structure be identified, this could inform policies. Conservation measures could avoid extinction of native races, ecotypes, and diversity to be found in these populations. Genetic similarity of donor and recipient populations may be considered in recommending migration routes for migratory beekeepers and bee sales.

The research herein tested hypotheses regarding the occurrence of recent admixture in Turkish honey bee populations across the subspecies of *A. m. syriaca*, *A. m. caucasica*, *A. m. anatoliaca*, and the C-lineage ecotype in Thrace using 30 microsatellite markers. In addition, we: (i) investigated the robustness of genetic diversity of honey bees in geographic areas where migratory beekeeping is restricted for varied reasons; (ii) compared patterns of genetic diversity of honey bees between migratory and stationary colonies; and (iii) determined the degree, origin, and direction of introgression in the Turkish honey bee populations to assess the consequences of unregulated queen and colony trade.

MATERIALS AND METHODS

Sampling

We sampled a single honey bee each from 250 colonies located in 18 Turkish provinces during the period of March 2010 through August 2012. Of the 250 honey bees sampled, 174 were from stationary and 76 from migratory colonies. Beekeepers who participated in this study declared that they used honey bees from stocks native to their area and that they had not purchased non-native queens or colonies in the last 10 years. Honey bee samples were stored at −80°C prior to genetic analysis.

We grouped samples from provinces with small sample sizes with nearby provinces to form 10 major localities: (1) Kırklareli; (2) Edirne + (Edirne and Tekirdağ); (3) Muğla; (4) Eskişehir + (Eskişehir, Kütahya, and Bilecik); (5) Düzce + (Düzce, Zonguldak, and Bolu); (6) Ankara; (7) Hatay; (8) Bitlis + (Bitlis, Elazığ, Erzurum, and Ordu); (9) Ardahan;

and (10) Artvin. The localities sampled correspond to the natural distribution range of the five subspecies that occur in Turkey: *A. m. syriaca* in Hatay, *A. m. caucasica* in Ardahan and Artvin, *A. m. anatoliaca* in Düzce, Eskişehir +, Muğla, and Ankara from the O lineage as well as the ecotype from the C subspecies group that occurs in Kırklareli and Edirne + and *A. m. meda*. Geographic locations were considered based on geographical proximity, and similarities in climate, topography, and floral profiles as well as preliminary data from previous studies. Sampling sites and sample sizes are listed in **Figure 1B**.

Genotyping

We isolated DNA from bee heads using the DNeasy Blood and Tissue Kit (Qiagen, Ankara) following the manufacturer's instructions, with slight modifications for insect samples. For polymerase chain reactions (PCR), we grouped a set of 30 microsatellite loci into four clusters for two 7-plex (set 1: AP218, A113, AB024, AP249, A088, AP001, AP043; set 2: AP049, AP238, AC006, AP243, AP288, HBC1602, A107) and two 8-plex (set 3: A079, AC306, AP226, A007, HBC1601, AP068, A014, AP223; set 4: AP019, AB124, A043, A076, AP273, AP289, HBC1605, A028) (Estoup et al., 1995; Solignac et al., 2003; Bodur et al., 2007; Shaibi et al., 2008; Tunca, 2009). The program, Multiplex Manager 1.2 (Holleley and Geerts, 2009) was used to determine the multiplex groups. Information on primer pairs, fluorescent dyes, and PCR conditions are provided in **Supplementary Tables 1, 2**.

The microsatellite allele sizes were determined by capillary electrophoresis with the ABI 3730XL sequencing machine (Applied Biosystems, Foster City CA). Locus A076 did not consistently amplify across samples; thus, it was excluded from the data and the downstream analysis.

Population Structure

We calculated pairwise F_{ST} values using Arlequin 3.5 (Excoffier and Lischer, 2010); the Mantel test with 10,000 permutations was used to test for isolation by distance. Pairwise population distances were calculated (Reynolds et al., 1983) using Populations 1.2.32 software (Langella, 2011) and visualized with the online tool Interactive Tree of Life v4 (Letunic and Bork, 2019). We used PAST4 and PCAgen software to plot relationships of populations on a two-dimensional space using a correlation matrix between groups (Goudet, 1999; Hammer et al., 2001).

Population structure was estimated by Structure 2.3.3 (Pritchard et al., 2000), *K*-values of distinct populations were analyzed by Structure Harvester software (Earl and von Holdt, 2012), and we used the Clumpp software (Jakobsson and Rosenberg, 2007) to permute the membership coefficients of individuals determined by Structure 2.3.3 and Distruct software (Rosenberg, 2004) to visualize the results obtained by Clumpp.

Other population genetic parameters and diversity indicators were calculated and include the frequency of null alleles, allelic richness and diversities, inbreeding and prevalence of close relatives, number of effective alleles, levels of heterozygosity, deviations from Hardy–Weinberg, linkage disequilibrium, bottlenecks, effective population sizes, and microsatellite information index (**Supplementary Tables 3–10**).

Statistical Analyses

To test the hypotheses regarding beekeeping practices, conservation sites, and queen/colony trade, we used membership coefficients. We first applied the arcsine square-root (angular) transformation to the coefficients since the data were composed of proportions and not normally distributed (Rohlf and Sokal, 1995). Then we performed Shapiro, Mann–Whitney *U*, Kruskal–Wallis, Dunn's, *F*, ANOVA, Tukey's, and *t*-tests wherever necessary and applicable to compare mean membership coefficients and estimated Cohen's *d* to determine effect sizes. The above tests were carried out with R statistical software using packages pwr, effsize, dunn.test, and dabestr (R Core Team, 2013; Torchiano, 2016; Dinno, 2017; Champely et al., 2018; Ho et al., 2019). The associated code is provided as **Supplementary Material** (R code).

Estimation plots were used to visualize untransformed data for membership coefficients and the impact of experimental factors. This is a less conventional method than bar or boxplots and the reporting of significance tests but more convenient and powerful to summarize all the data in an unbiased manner by displaying all measurements and effect sizes as well as the precision of estimates and distribution of mean differences (Ho et al., 2019).

Beekeeping Practice: Migratory vs. Stationary

To test the hypothesis of whether beekeeping practice affects population structure and subspecies identity, we compared membership coefficients of migratory and stationary colonies in Ankara, Muğla, and Hatay separately, combined, and for the total data set. We propose that if migratory colonies acted as a potential vector of foreign honey bee alleles, then samples would have much lower probabilities of being assigned to the clusters of origin.

We used all samples ($N = 250$) to quantify the differences in membership coefficients for migratory and stationary colonies. For the remaining analysis, we used a subset of the samples from stationary colonies ($n = 174$) since this can give a better perspective of the population structure.

Isolated Regions as Conservation Sites

If isolated regions preserve genetic diversity by preventing gene flow, we predict higher membership coefficients for samples that originate from isolated regions compared with those from regions exposed to migratory beekeeping.

Kırklareli is officially declared as an isolated region. This is due to local beekeepers' long-standing negative attitude and resistance to migratory beekeepers. As a result, they have not accessed this region for many years. This region is home to a C-lineage honey bee ecotype, carefully maintained by local beekeepers. Ardahan is legally declared as a conservation and breeding area for *A. m. caucasica*, therefore migratory beekeepers cannot enter the province, and queen import from other subspecies is forbidden. Parts of Artvin province are also officially declared as isolated regions for the conservation of *A. m. caucasica* as a pure race. The province, in general, is rarely visited by migratory beekeepers because of the difficulties in transportation in the rough terrain. Moreover, local beekeepers often engage in commercial queen sales so, they only use native-bred queens. We compared the above three provinces with restricted inflow of migratory beekeepers with the other six regions (Edirne +, Muğla, Düzce +, Eskişehir +, Ankara, and Hatay) where migratory beekeeping and bee trade are freely exercised.

Effect of Queen and Colony Trade

Using all samples, we compared membership coefficients in non-native clusters between each other to determine which groups contributed most to other populations' gene pools.

Ardahan and Artvin provinces host the *A. m. caucasica* subspecies, which is widely used for commercial purposes. *A. m. caucasica* queens and their hybrids are sold throughout Turkey. However, these provinces are limited to a very narrow range in the Northeast of the country and are declared isolated regions. Therefore, a high introgression of *caucasica* alleles from these regions would mostly, if not completely, be due to the replacement of queens and colonies.

We also tested for the presence of other genetic patterns within the Turkish honey bee population to understand the magnitude and direction of gene flow within and across the sampled localities.

RESULTS

Population Genetic Structure

We calculated F_{ST} values by using the frequencies obtained in the study and the null allele corrected frequencies. We calculated an F_{ST} of 0.065 for all samples and an F_{ST} of 0.067 after correction for the stationary colonies ($n = 174$). The F_{ST} values for migratory colonies were 0.011 and 0.015, respectively, and for all the 250 samples, they were 0.046 and 0.047. We plotted stationary colonies on 2D space by carrying out the principal component analysis (PCA) (**Figure 2A**). The x and y axes explained, respectively, 41.8 and 32.1% of the variance within the samples. For stationary colonies, the phylogenetic tree constructed using pairwise population distances resolved four distinct branches (**Figure 2B**). Using the Structure Harvester clustering program, we determined that $K = 2$ and $K = 4$ gave similar outcomes with the latter being more likely as this mirrors the number of subspecies present in the regions sampled.

We calculated membership coefficients of individuals to the observed clusters in $K = 4$ and found no population structure for migratory colonies (**Figure 3A**) in contrast to samples from stationary colonies and the entire data set (**Figures 3B,C**).

Effects of Beekeeping Practices and Conservation

Results from the Mantel test showed a significant correlation with geographic distance between populations ($r = 0.60$, $p < 0.01$) for stationary but not migratory colonies. Distance matrices and test results are provided in **Supplementary Tables 11, 12**.

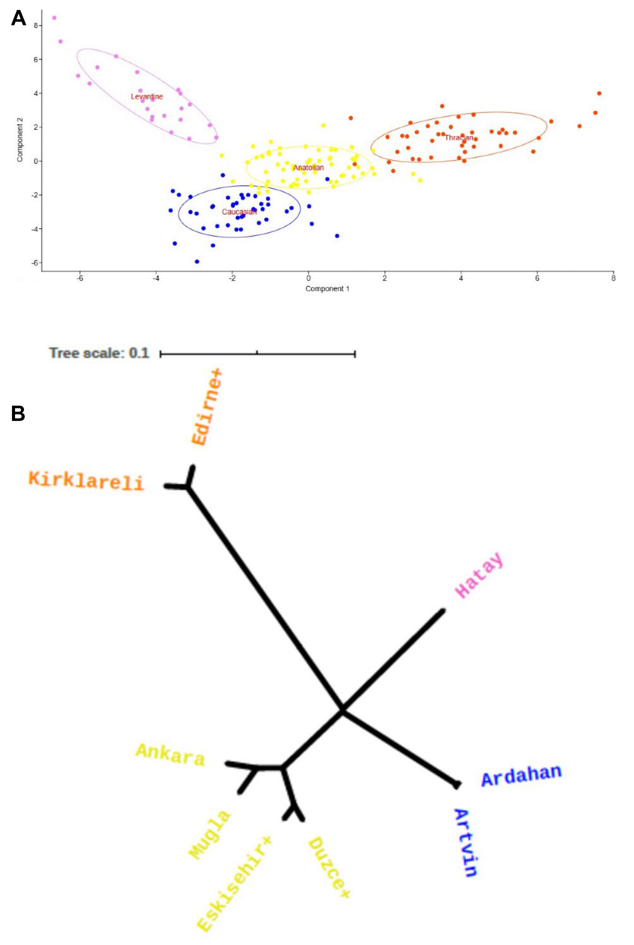

FIGURE 2 | (A) Principal component analysis (PCA) of stationary colonies with 66% concentration ellipses shown. Component 1 and Component 2 explain 41.8 and 32.1% of the variance within the samples, respectively. The first axis differentiated samples in Thrace indicating strong divergence between those and others whereas the second axis differentiated subspecies throughout Anatolia. Each dot represents an individual (orange: C-lineage subspecies in Thrace; O-lineage subspecies: yellow: *anatoliaca*, blue: *caucasica*, violet: *syriaca*). **(B)** UPGMA tree of honey bee populations based on Reynolds, Weir, and Cockerham's genetic distances. Tree resolves four distinct branches corresponding to four subspecies. Thracian populations constitute the extreme end of the unrooted tree. The other end is divided into three almost equidistant branches of Caucasian, Levantine, and Anatolian populations.

A significant difference was detected in a comparison of membership coefficients of individuals from stationary and migratory colonies (**Figure 4A**). Stationary colonies from Muğla and Hatay had a higher likelihood to be assigned to their own clusters than migratory colonies sampled from these provinces ($p < 0.05$ and $p < 0.001$, respectively, Mann–Whitney *U* and *t*-tests). The same pattern was observed when the combined data from the three provinces ($p < 0.01$), or all the migratory and stationary colonies ($p < 0.001$) were considered. However, the situation was reversed in Ankara ($p < 0.05$). In all the comparisons but one, 95% CI of the mean differences between the membership coefficients of migratory and stationary colonies lie

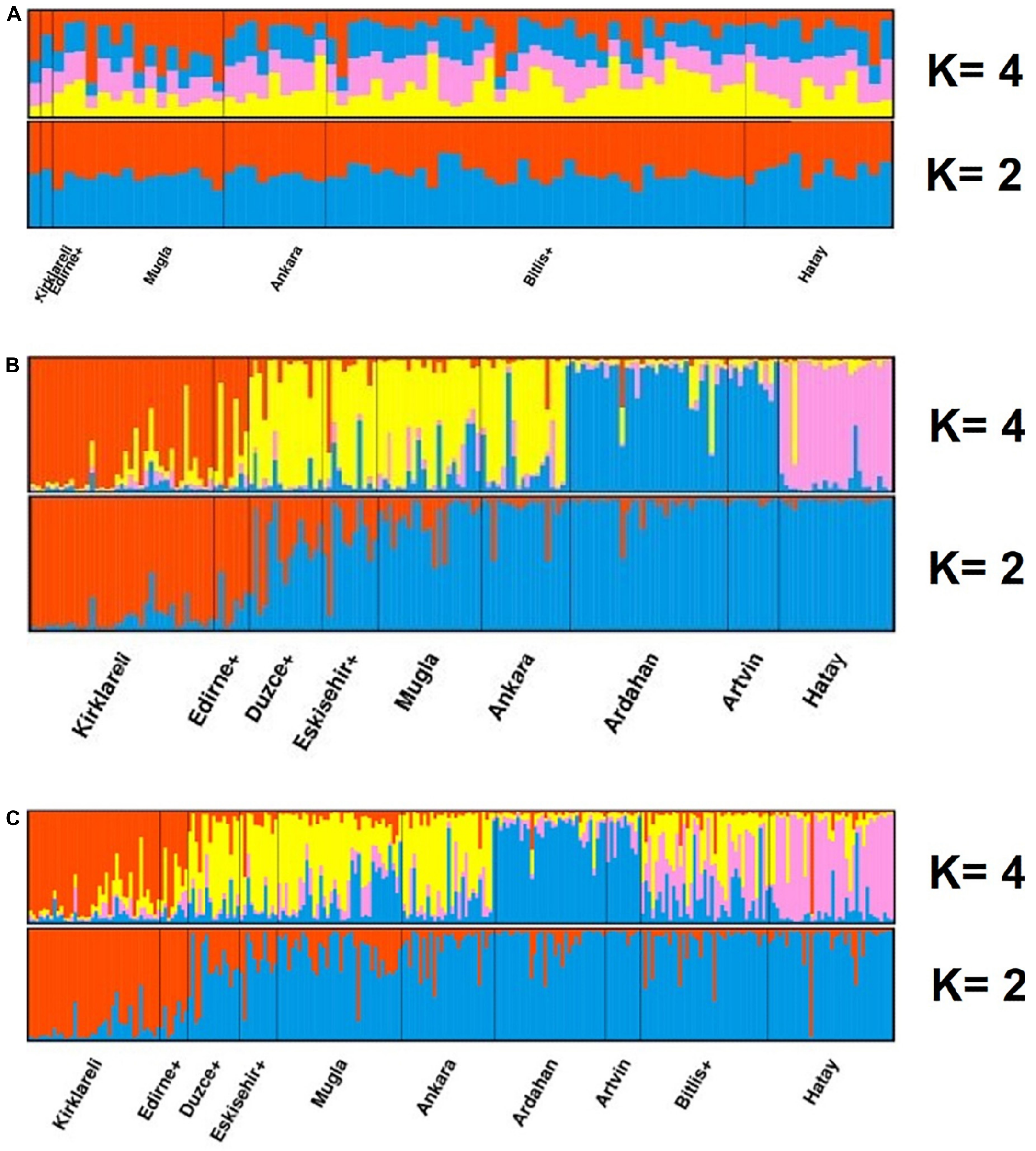

FIGURE 3 | Estimated population structure and clustering of honeybees in Anatolia and Thrace for **(A)** migratory colonies, **(B)** stationary colonies, and **(C)** the whole sample. Structure analysis is based on microsatellite data and suppose either $K = 2$ (orange: C-lineage, blue: O-lineage) or $K = 4$ (orange: Thracian, yellow: Anatolian, blue: Caucasian, violet: Levantine) hypothetical populations. Each individual is represented by a vertical bar and colored according to membership coefficients belonging to each cluster. $K = 2$ is found to be slightly likelier than $K = 4$. For stationary colonies at $K = 2$, the transition from C-lineage to O-lineage is gradual. No population structure is observed in migratory colonies in contrast to stationary ones and the overall data where four different subspecies are evident. Note the higher admixture levels in the overall data in comparison to stationary colonies.

below the zero-line (**Figure 4B**). The mean values, effect size, and significance level of the differences are summarized in **Table 1**.

The comparison of isolated regions with those open to migratory beekeeping (**Table 1** and **Figure 5A**) showed that stationary colonies within isolated regions have significantly higher fidelity to their original clusters ($p < 0.001$, Mann–Whitney *U* and *t*-tests). This can also be seen in the estimation plot (**Figure 5C**) where the mean membership coefficients of samples that are from regions open to migratory beekeeping are lower and fall beyond the 95% confidence interval of the estimated mean of the difference between the two groups. In addition, despite the lack of conservation efforts, samples from Hatay and Düzce + showed membership coefficients comparable with those of Kırklareli, Ardahan, and Artvin (**Figure 5B**).

Impact of Queen and Colony Trade

If an individual is assigned with high probability to its own cluster, i.e., 90% probability, there remains a 10% chance that

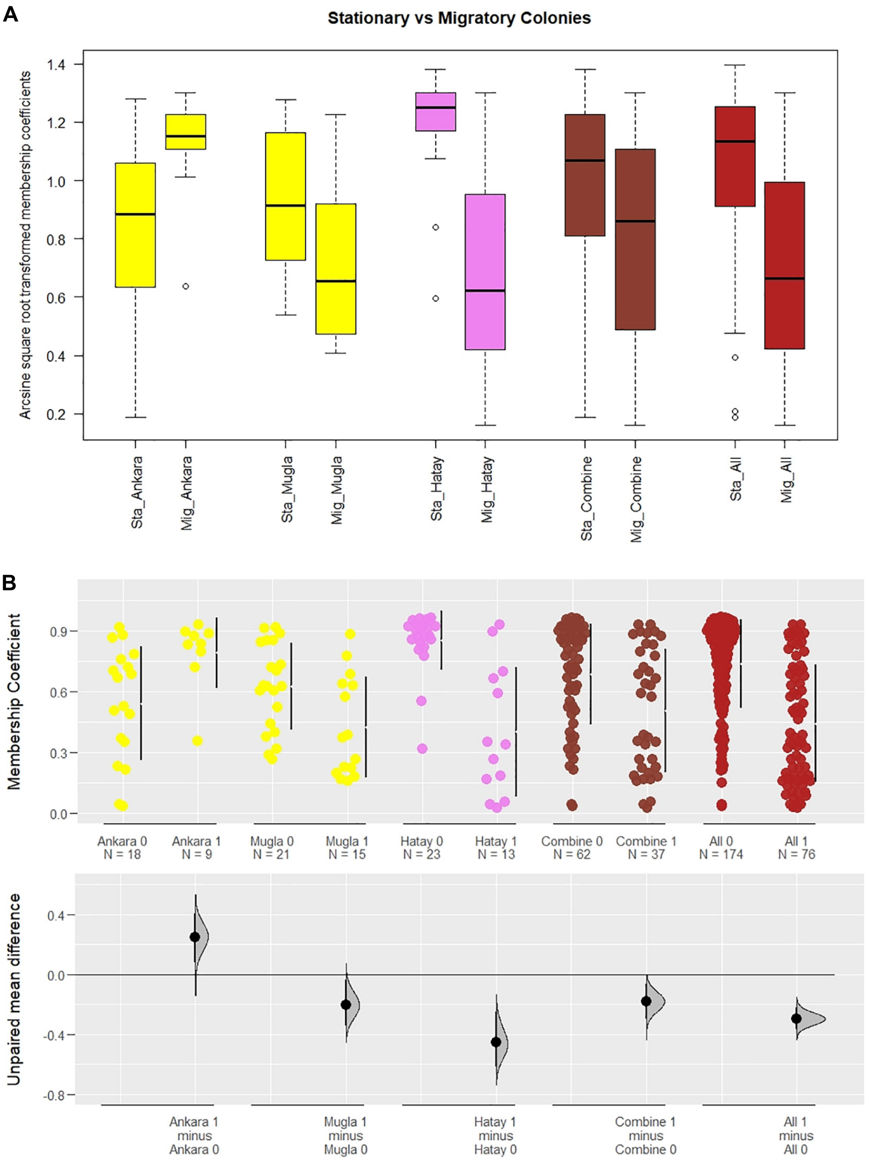

FIGURE 4 | Comparison between stationary (Sta_) and migratory (Mig_) colonies in Ankara ($p < 0.05$), Muğla ($p < 0.05$), and Hatay ($p < 0.001$), as well as these three provinces combined ($p < 0.01$) and the whole data set ($p < 0.001$). **(A)** Boxplot display of arcsine square-root transformed membership coefficients used in significance testing of comparisons. **(B)** Scatter plot with estimations of mean differences based on raw individual membership coefficients (yellow: Ankara and Muğla belonging to the Anatolian cluster, violet: Levantine cluster, coral: for a combination of three provinces, firebrick: whole data). Stationary colonies are annotated as < Group name > 0 and migratory colonies as < Group name > 1. Bars right to the data points refer to the 25 and 75% quartiles and the gap between them is the median value for the sample. The zero-line below corresponds to the mean membership coefficients of stationary colonies in each pairwise comparison. The Euclidean distances from those means for the migratory colonies are shown as dots with a 95% confidence interval bar around. Also, distributions of the estimation statistics are included to comprehensively compare the strength of the drift for different populations and subsets of the data. Stationary colonies exhibit higher mean membership coefficients than migratory ones except for Ankara where the vice versa is true.

it can be assigned to other clusters. Given four clusters, we investigated whether these mis-assignment probabilities were enriched for any particular cluster. The mean transformed values of cluster mis-assignments among individuals of other populations were as follows: Thracian 0.16, Anatolian 0.25, Caucasian 0.26, and Levantine 0.20 (**Figure 6A**).

A significant Kruskal–Wallis test ($p < 0.001$) and a *post hoc* Dunn's test, accompanied by a significant ANOVA result ($p < 0.001$) followed by a Tukey's test, showed that mis-assignments to *A. m. caucasica* and *A. m. anatoliaca* clusters were significantly more frequent than to the other subspecies ($p < 0.001$ for both subspecies against C-lineage Thracian bees and $p < 0.05$ against *syriaca* group). The effect sizes according to Cohen's *d* varied from 0.34 to 0.54 with estimation plots verifying the precision of the difference observed (**Figure 6B**). Despite the observation of the highest values in *A. m. caucasica*

TABLE 1 | Genetic impact of beekeeping and conservation practices on (arcsine square-root transformed) membership coefficients to native clusters (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$).

	n Migratory	n Stationary	Stationary	Migratory	Cohen's *d*	*U* and *t*-test significance
Beekeeping practice						
Ankara	9	18	0.82	1.11	−1.00	*
Muğla	15	21	0.93	0.70	0.89	*
Hatay	13	23	1.20	0.66	2.01	***
Combined	37	62	1.00	0.79	0.66	**
Overall	76	174	1.06	0.72	1.22	***
Conservation practice						
Isolated	NA	79	1.21	NA	0.49	***
Not-isolated	NA	95	1.08	NA		

mis-assignments, the results between *A. m. caucasica* and *A. m. anatoliaca* clusters were not significant. We tested whether these differences were due to many individuals with high admixture levels, but such data only constituted 7.5% of all the observations. This figure is obtained by a threshold of 0.5 for the transformed values, which corresponds to a second hybrid, implying a 25% contribution of non-native origin.

We also investigated if these small drifts in admixture proportions were more prominent in some localities and if populations differed as to the identity of the subspecies from which they receive gene flow. This led us to learn the extent, magnitude, and direction of gene flow among the subspecies with a particular sensitivity to specific populations (**Figure 7**). Results of Dunn's test for each pairwise comparison between populations are in **Supplementary Tables 13–16** (12 significant differences out of 80 comparisons in total).

DISCUSSION

Given the promiscuous nature of the honey bee mating system, it has been suggested that large-scale migratory beekeeping and bee trade have exposed local populations to introgression (De la Rúa et al., 2009). Although there is evidence that management actually increased genetic diversity (Harpur et al., 2012), admixture can also drive the loss of valuable local adaptations (De la Rúa et al., 2013). Since the global environment alters with an increasing pace, honey bees face new challenges in which they need to rely on adaptations and genetic diversity they accumulated over the course of their evolutionary history (Kükrer and Bilgin, 2020).

The main finding of this study is that there are distinct populations of subspecies of bees, isolated by distance, yet migratory colonies and bee trade likely cause gene flow across these populations in Turkey. The differences in F_{ST} values between stationary and migratory colonies indicate that the latter, with lower F_{ST} values, experience a high degree of gene flow. This conclusion is also reflected by the absence of positive correlation between genetic and geographic distances in migratory colonies in contrast to stationary colonies where an isolation by distance pattern was observed (**Supplementary Tables 11, 12**). Overall, F_{ST} values obtained were highly significant but lower than those from Bodur et al. (2007), estimated for samples collected 10 years prior to the study herein, that showed total levels of F_{ST} of 0.077 together with higher values for pairwise comparisons among populations. This may indicate recent increased gene flow and may signal an alarming trend toward greater movement of honey bees in the regions sampled. Long-term studies are needed to determine if this is a persistent trend.

Structure of Bee Populations in Turkey

PCA results confirmed the four different clusters inferred from the UPGMA tree topology (**Figure 2**). The first axis designating the first principle component differentiated Thracian samples, whereas the second axis, corresponding to the second component differentiated subspecies in Anatolia (*syriaca*, *anatoliaca*, and *caucasica*). Bitlis + samples clustered with Central and West Anatolian populations in both phylogenetic tree and PCA results (**Supplementary Figures 1, 3, 4**). However, all samples from this locality were from migratory colonies thus resampling this area with the inclusion of stationary colonies from East Anatolia would render a clearer picture of the phylogenetic relationship of these populations.

The two most likely *K*-values in structure analysis for all samples and the stationary colonies were $K = 2$ and $K = 4$, both results support the hypotheses of the sampled populations belonging to two separate lineages (C and O) in line with (Kandemir et al., 2005) and four distinct subspecies (a Carniolan ecotype in Thrace, *A. m. caucasica* in Artvin and Ardahan, *A. m. syriaca* in Hatay and *A. m. anatoliaca*, widely distributed, covering the rest of the country) (**Figure 3**). In contrast to the belief that migratory beekeepers make use of native stocks for their operations, our results showed the absence of structuring in these samples, and support the conclusion that migratory apiaries are highly hybridized.

Distinct Phylogeographic Patterns in Stationary Bees

Stationary apiaries, as expected, yielded highly structured groups where all the subspecies could be detected. When K was 2, the structure analysis of two distinct clusters showed that there was a transition zone between Thracian and Anatolian samples around the Marmara Sea and Aegean. Ruttner's analysis based on morphometry (1988) distinguished bees in Western Anatolia from the rest of the *anatoliaca* group. Contributions from the Thracian cluster are significantly high in Düzce + and

Eskişehir + located southeast of the Sea of Marmara across the Bosporus. Also, there are some non-significant overabundant Thracian contributions in Muğla province on the Aegean coast (**Figure 3**). This may constitute a hybrid zone between the C and O lineages and resemble the hybrid zones identified between M and C lineages in the Alps and the Apennine Peninsula and between A and M lineages in the Iberian Peninsula and Mediterranean islands (De la Rúa et al., 2009). An expected symmetrical introgression might be the reason behind the East–West cline observed by Muñoz and De la Rúa (2020) in four distinct ecotypes of *A. m. carnica* and *A. m. macedonica* in the Balkan Peninsula.

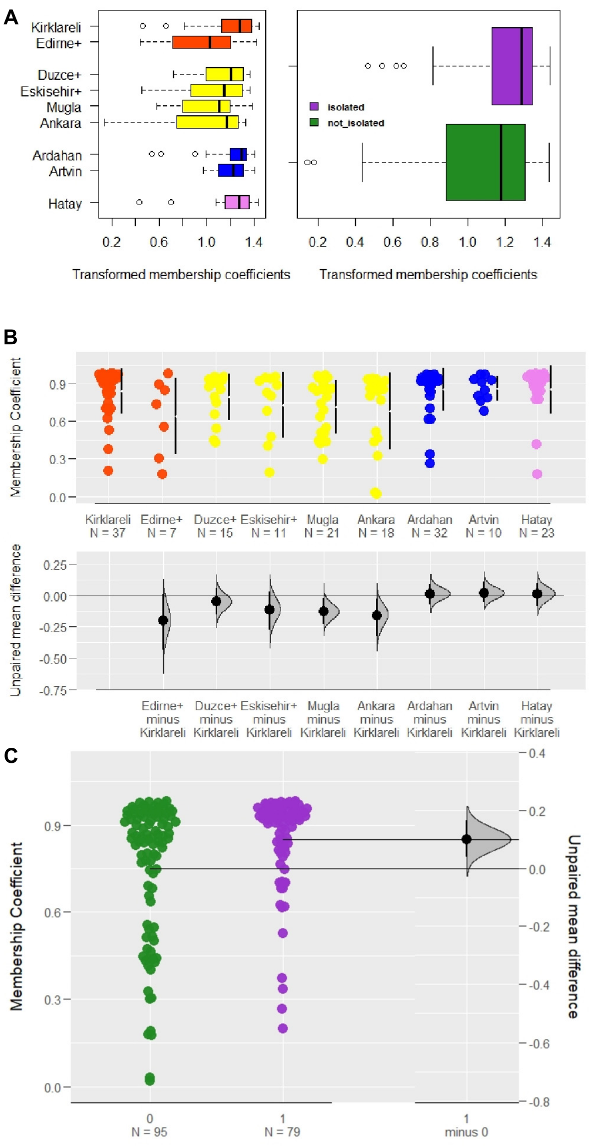

FIGURE 5 | Samples within isolated regions assigned to their clusters with higher probabilities in contrast to samples from regions open to migratory beekeeping. **(A)** The first boxplot displays the arcsine square-root transformed membership coefficients for nine populations, whereas the second one presents a comparison of samples within isolated regions and those are not ($p < 0.001$). **(B)** Scatter plot based on a comparison of raw individual membership coefficients to their native clusters for nine populations against Thracian samples. Bars right to the data points refer to the 25 and 75% quartiles and the gap between them is the median value for the sample. Note that despite lacking a conservation status, samples from Hatay and Düzce + have membership coefficients comparable to those of Kırklareli, Ardahan, and Artvin which are isolated regions. **(C)** Scatter plot contrasting individual raw membership coefficients with an estimation of the mean difference between isolated regions and those are not (orange: Thracian, yellow: Anatolian, blue: Caucasian, violet: Levantine clusters; orchid and "1": isolated regions, green and "0": regions open to migratory beekeeping). The zero-line corresponds to the mean membership coefficient of colonies in regions open to migratory beekeeping. The Euclidean distance of the colonies in isolated regions from that mean is shown as a dot with a 95% confidence interval bar around. The distribution of the estimation statistic is included to account for the precision.

When K was considered as 4, all four subspecies were easily differentiated from each other. The significance of two distinct clusters ($K = 2$) was higher than four ($K = 4$) indicating the evolutionarily greater differences between the lineages belonging to C (in Thrace) and O (in Anatolia).

Thracian samples form a clade in the unrooted phylogenetic tree while the other three populations, Caucasian, Levantine, and Anatolian are equidistant from each other and form a separate clade. These results indicate that the Thracian population is distinct from the others and likely has experienced limited gene flow in allopatry, supporting the hypothesis for a Carniolan (C-lineage) descent of Thracian bees in Turkey. A direct comparison with honey bee samples of the major C-lineage subspecies would confirm the subspecies of these bees which are highly differentiated from Anatolian samples. This finding is in contrast to the conclusions of Ruttner (1988) that Thracian bees are part of the *anatoliaca* subspecies groups and merits further investigation.

A. m. anatoliaca samples formed a distinct cluster in structure analysis, yet fell in the middle of the other subspecies in ordinations according to F_{ST} values. This similarity may point to a significant historical contribution to *A. m. anatoliaca* populations from the neighboring regions. Another explanation is that the putative basal position of *anatoliaca* for O-lineage honey bees places this group at the center of genetic diversity. In contrast to *anatoliaca* bees forming a distinct group, all-migratory Bitlis + samples were a mixture of different clusters and did not form a separate group.

A greater understanding of phylogenetic relationships of the populations of bees in Turkey can be achieved only if neighboring populations in the Balkans, Iran, Caucasus, and Southwest Asia are also sampled. This future research direction may clarify the complex taxonomic relations within and between the C and O lineages, and delimit distributions and transition zones of the subspecies in this region.

Homogenizing Effect of Migratory Beekeeping

Migratory colonies are acting as a hybrid zone mobile in space and time. The colonies are in one region in spring and in others in summer and fall. As such, these bees serve as vectors of otherwise local gene combinations. Statistical comparison of migratory and stationary colonies confirms the significant gene flow toward the migrants from local bees (**Figure 4**). Likewise, a significant gene

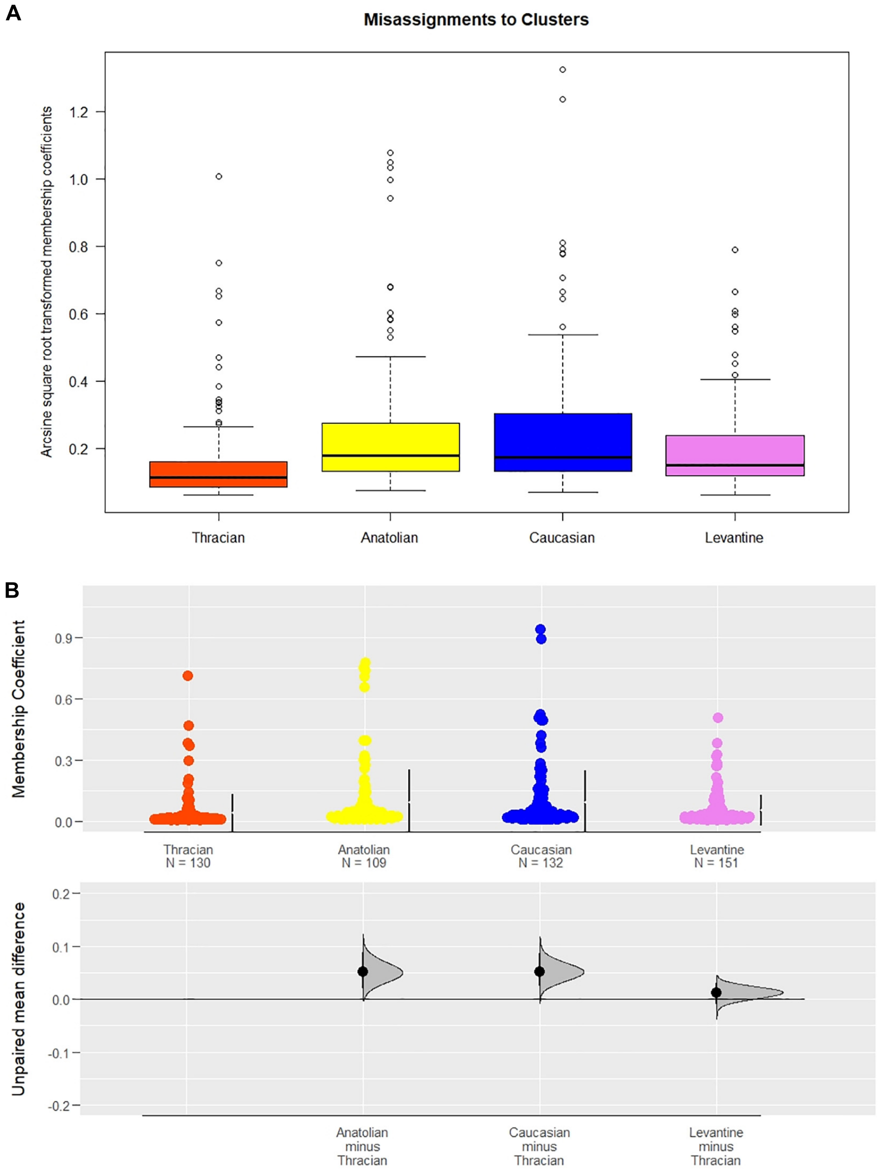

FIGURE 6 | Mis-assignment of individuals to *caucasica* and *anatoliaca* clusters were significantly more frequent than the others ($p < 0.001$ for both subspecies against C-lineage Thracian bees and $p < 0.05$ against *syriaca* group). **(A)** Boxplot display of arcsine square-root transformed membership coefficients mis-assigned to each cluster. **(B)** Scatter plot with estimations of mean differences against Thracian mis-assignments based on raw individual membership coefficients (orange: C-lineage Thracian cluster, yellow: *anatoliaca*, blue: *caucasica*, violet: *syriaca*). Bars right to the data points refer to the 25 and 75% quartiles and the gap between them is the median value.

flow toward local stationary bees was also observed outside the conservation sites. These results, derived from direct comparison of two distinct contrasts, demonstrate the vitality of establishing areas away from migratory beekeeping for the preservation of honey bee genetic diversity. This conclusion is in agreement with other studies on conservation practices (Oleksa et al., 2011; Pinto et al., 2014).

An exception that proves the point is the lower assignment probability of bees sampled from Ankara to their province, even in comparison to migratory bees in the same location. There are two factors: First, the region's beekeepers prefer to use queen bees native to the region. The second factor is that this region is a principal queen breeding area. The Kazan apiary of TKV (Development Foundation of Turkey) uses hundreds of colonies of Caucasian bees and raised queens are sold around the country for over 30 years. Many independent queen bee breeders in the Kazan region continue the same practice. Gene flow from these queen breeders' apiaries may contribute to the admixture observed in stationary colonies in Ankara. The high mis-assignment probability of colonies in Ankara to the Caucasian cluster supports this hypothesis.

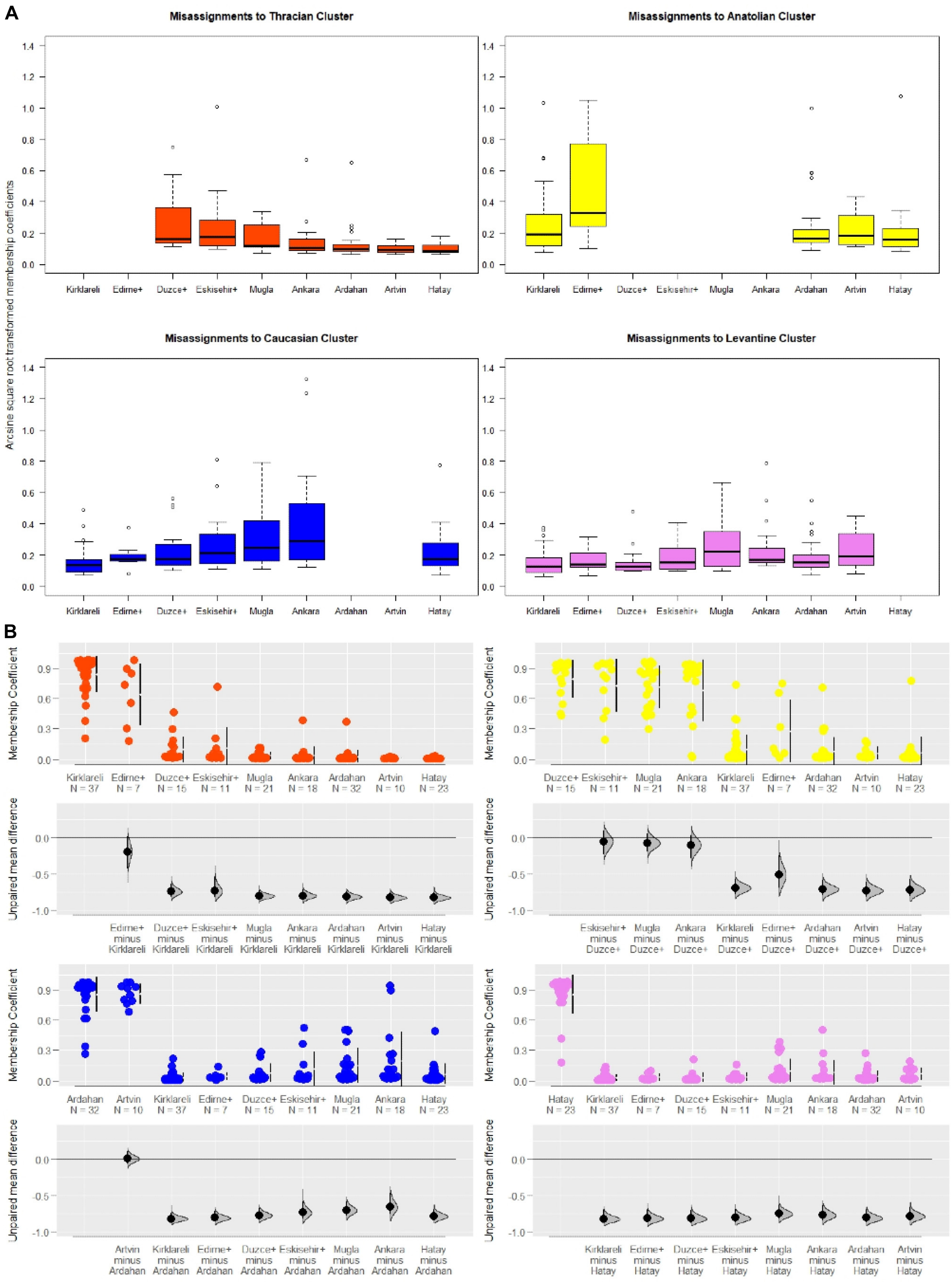

FIGURE 7 | Patterns of gene flow between populations. **(A)** Boxplot displays of arcsine square-root transformed membership coefficients mis-assigned to each cluster (12 significant differences out of a total of 80 comparisons are provided in the **Supplementary Material**). **(B)** Scatter plots with estimations of mean differences based on raw individual membership coefficients to each cluster (orange: Thracian, yellow: Anatolian, blue: Caucasian, violet: Levantine clusters) contrasted against Kırklareli, Düzce +, Ardahan, and Hatay populations representative of four subspecies. Bars right to the data points refer to the 25 and 75% quartiles and the gap between them is the median value.

Direction and Magnitude of Introgression Determined by Bee Trade

It is hard to directly quantify the effect of queen and colony trade on genetic mixing. The availability of several naturally occurring subspecies in Anatolia and Thrace helps in understanding the relative role of queen and colony trade in gene flow. Honey bees from stationary colonies were assigned more often to their native clusters, yet they were also assigned to other clusters with lower probabilities. Samples in the whole range of the study mis-assigned to the Caucasian cluster more often than they were mis-assigned to others (**Figure 6**). This is most likely due to the wide distribution of Caucasian queen bees by queen trade.

Migratory beekeeping is not practiced in Ardahan and Artvin where highly commercial Caucasian bees are native. Hence, no bees go in or leave out the region as migratory colonies. We infer that the observed introgression of Caucasian alleles to the stationary colonies elsewhere could mainly be attributed to the frequent purchase of *caucasica* queen bees and colony replacements in neighboring apiaries within those regions. Practices of neighboring beekeepers become important because even if beekeepers included in this study within a region do not purchase *caucasica* queens, their colonies may be subject to queen supersedure and natural mating in that region.

Central and western Anatolian populations suffer heavily from gene flow from Caucasian populations as demonstrated by our results (**Figure 7**). Muğla, which receives millions of migratory colonies during the honeydew season, and Ankara showed high levels of significant gene flow from other subspecies, especially the *caucasica*. This is especially alarming because Muğla (in the southwest) and the Caucasus region (in the northeast) lie at the diagonal extremes of the country, some 1,500 km apart.

A. m. anatoliaca alleles also showed high introgression especially in the Thrace region but also at average levels in other regions. These high levels may be related to the geographical proximity of this subspecies to other populations. The proximity may explain historical and recent gene exchanges. Alternatively, widespread practice of migratory beekeeping by Western and Central Anatolian beekeepers throughout Turkey may have contributed to observed introgression. In this case, queen replacement could be a minor contributor since there are very few commercial queen breeders within the distribution range of *A. m. anatoliaca*.

Conservation Sites

The importance of establishing isolated regions was highlighted with genetic data. The results of the statistical tests showed a significant difference between the conservation of identity in and out of isolated regions with isolated regions staying purer in terms of subspecies composition (**Figure 5**). Such regions were proven to be effective in the conservation of unique diversity present within (Requier et al., 2019).

In the light of this study, we propose a renewed effort to address the need for massive establishing of such regions for conserving locally adapted native bees throughout the whole natural distribution of the species. This especially holds for underrepresented regions in terms of local diversity hotspots. A gap analysis aiming for complementarity in the planning of systematic conservation efforts is urgently needed globally.

In such isolated regions, naturally, migratory beekeeping, as well as replacement of queen bees with non-native ones, must be strictly prohibited and checked by relevant molecular monitoring techniques. However, these isolated regions should also be wide enough involving additional buffer zones where further restrictions on migratory beekeeping and bee trade are applied for efficient isolation and for fulfilling sufficient effective population sizes.

Thanks to increasing awareness in the last decade within the industry, now there are at least 11 isolated regions in service or being established in Turkey. These conservation sites make ideal places for breeding purposes. The establishment of such sites is achieved through the significant efforts of scientists and their collaboration with the Turkish Beekeepers Association (Kükrer and Bilgin, 2020). There is an ever-growing need for establishing closer links with decision makers and stakeholders and the necessity of investing more efforts in communicating the results of scientific studies to all involved.

CONCLUSION

Overall results of this study clearly show that the genetic structure of honeybee populations in Turkey is highly conserved. This, however, does not mean that the structure and the diversity observed are secure. Rather the honey bee genetic diversity in Turkey should be considered under threat. We demonstrated continued gene flow and admixing of populations, likely due to anthropogenic factors.

The preservation of population structure despite movement of the high number of colonies and unregulated and frequent queen and colony sales is biologically interesting. Future research may also need to focus on how this biodiversity and its structuring were preserved and its relation to natural selection. The relative effects of natural selection and gene flow should be compared; the former could significantly counterbalance the latter.

Genetic variation eventually leading to local adaptations with such a significant outweighing effect can be considered as a valuable resource for honey bee populations in the global context at this time of unusual bee losses as well as global climate change. A better understanding of present adaptation to both local climate and geographic conditions as well as adaptive capacity to future changes is important for bees and stakeholders. A fair amount of effort should be invested in more studies focusing on candidate functional variants at the genome level that play role in due process in different parts of the world. Novel and innovative ways of coping with environmental and climatic stressors developed by honey bee populations or exploration of interesting patterns of convergent evolution are waiting ahead to be yet discovered.

Our overall results are restricted to the present situation of honey bee subspecies in Turkey, yet they highlight the significance of local populations and provide a preliminary quantification of human impact. We expect our findings on migratory beekeeping, trading of queens and colonies as well as conservation implications to be of use for the decision makers and other stakeholders.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

MKü carried out the experiments and statistical analyses and took the lead in writing the manuscript. AK was in charge of overall direction and planning. All authors conceived and planned the experiments and contributed to the fieldwork, contributed to the interpretation of the results, provided critical feedback and helped shape the research, analysis, and manuscript.

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DEDICATION

This article is dedicated to the memory of the late AK who spent his last 20 years studying honey bee diversity in Turkey trying strongly to draw the attention of researchers, decision makers, and beekeepers to the conservation of locally adapted native bees as a precious legacy of our kind. He will be remembered also for defending the theory of evolution to be taught in the science curriculum and for training many valuable young evolutionary biologists under very harsh conditions. He passed away on February 1, 2014 after the completion of the study and at the very beginning of the manuscript preparation.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.556816/full#supplementary-material

REFERENCES

Akkaya, H., and Alkan, S. (2007). Beekeeping in Anatolia from the Hittites to the present days. *J. Apicultural Res.* 46, 120–124. doi: 10.3896/ibra.1.46.2.10

Blacquiere, T., Smagghe, G., Van Gestel, C. A., and Mommaerts, V. (2012). Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment. *Ecotoxicology* 21, 973–992. doi: 10.1007/s10646-012-0863-x

Bodur, C., Kence, M., and Kence, A. (2007). Genetic structure of honey bee, Apis mellifera L. (Hymenoptera:apidae) populations of Turkey inferred from microsatellite analysis. *J. Apicultural Res.* 46, 50–56. doi: 10.1080/00218839.2007.11101366

Bouga, M., Alaux, C., Bienkowska, M., Buchler, R., and Carreck, N. L. (2011). A review of methods for discrimination of honey bee populations as applied to European beekeeping. *J. Apicultural Res.* 50, 51–84.

Breeze, T. D., Bailey, A. P., Balcombe, K. G., and Potts, S. G. (2011). Pollination services in the UK: how important are honey bees? *Agric. Ecosyst. Environ.* 142, 137–143.

Brown, M. J. F., and Paxton, R. J. (2009). The conservation of bees: a global perspective. *Apidologie* 40, 410–416. doi: 10.1051/apido/2009019

Büchler, R., Costa, C., Hatjina, F., Andonov, S., Meixner, M. D., Conte, Y. L., et al. (2014). The influence of genetic origin and its interaction with environmental effects on the survival of Apis mellifera L. colonies in Europe. *J. Apicultural Res.* 53, 205–214. doi: 10.3896/IBRA.1.53.2.03

Cánovas, F., de la Rúa, P., Serrano, J., and Galián, J. (2011). Microsatellite variability reveals beekeeping influences on Iberian honeybee populations. *Apidologie* 42, 235–251. doi: 10.1007/s13592-011-0020-1

Champely, S., Ekstrom, C., Dalgaard, P., Gill, J., Weibelzahl, S., Anandkumar, A., et al. (2018). *Package 'pwr'. R package version, 1–2.*

Cridland, J. M., Tsutsui, N. D., and Ramírez, S. R. (2017). The complex demographic history and evolutionary origin of the western honey bee, Apis Mellifera. *Genome Biol. Evol.* 9, 457–472. doi: 10.1093/gbe/evx009

Dall'Olio, R., Marino, A., Lodesani, M., and Moritz, R. F. (2007). Genetic characterization of Italian honeybees, Apis mellifera ligustica, based on microsatellite DNA polymorphisms. *Apidologie* 38, 207–217. doi: 10.1051/apido:2006073

De la Rúa, P., Jaffé, R., Dall'Olio, R., Muñoz, I., and Serrano, J. (2009). Biodiversity, conservation and current threats to European honey bees. *Apidologie* 40, 263–284.

De la Rúa, P., Jaffé, R., Muñoz, I., Serrano, J., Moritz, R. F., and Kraus, F. B. (2013). Conserving genetic diversity in the honeybee: comments on Harpur et al. (2012). *Mol. Ecol.* 22, 3208–3210. doi: 10.1111/mec.12333

Dietemann, V., Pirk, C. W. W., and Crewe, R. (2009). Is there a need for conservation of honey bees in Africa? *Apidologie* 40, 285–295.

Dinno, A. (2017). *Package 'dunn. test'. CRAN Repos.*

Earl, D. A., and von Holdt, B. M. (2012). Structure Harvester: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Resour.* 4, 359–361. doi: 10.1007/s12686-011-9548-7

El-Niweiri, M. A., and Moritz, R. F. (2010). The impact of apiculture on the genetic structure of wild honeybee populations (Apis mellifera) in Sudan. *J. Insect Conserv.* 14, 115–124. doi: 10.1007/s10841-009-9231-4

Estoup, A., Garnery, L., Solignac, M., and Cornuet, J. M. (1995). Microsatellite variation in honey bee (Apis mellifera L.) populations: hierarchical genetic structure and test of the infinite allele and stepwise mutation models. *Genetics* 140, 679–695. doi: 10.1093/genetics/140.2.679

European Parliament (2017). *The EU's Beekeeping Sector. At a Glance*. Available online at: https://www.europarl.europa.eu/RegData/etudes/ATAG/2017/608786/EPRS_ATA%282017%29608786_EN.pdf (accessed April 28, 2020)

Evans, J. D., and Schwarz, R. S. (2011). Bees brought to their knees: microbes affecting honey bee health. *Trends Microbiol.* 19, 614–620. doi: 10.1016/j.tim.2011.09.003

Excoffier, L., and Lischer, H. E. L. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* 10, 564–567. doi: 10.1111/j.1755-0998.2010.02847.x

Genersch, E. (2010). Honey bee pathology: current threats to honey bees and beekeeping. *Appl. Microbiol. Biotechnol.* 87, 87–97. doi: 10.1007/s00253-010-2573-8

Goudet, J. (1999). *PCAGEN, A Computer Package Which Performs Principal Component Analysis (PCA) on Gene Frequency Data.* Lausanne: Université de Lausanne.

Hammer, Ø, Harper, D. A. T., and Ryan, P. D. (2001). PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4:9.

Han, F., Wallberg, A., and Webster, M. T. (2012). From where did the Western honeybee (Apis mellifera) originate? *Ecol. Evol.* 2, 1949–1957. doi: 10.1002/ece3.312

Harpur, B. A., Kent, C. F., Molodtsova, D., Lebon, J. M., Alqarni, A. S., Owayss, A. A., et al. (2014). Population genomics of the honey bee reveals strong signatures of positive selection on worker traits. *Proc. Natl. Acad. Sci. U.S.A.* 111, 2614–2619. doi: 10.1073/pnas.1315506111

Harpur, B. A., Minaei, S., Kent, C. F., and Zayed, A. (2012). Management increases genetic diversity of honey bees via admixture. *Mol. Ecol.* 21, 4414–4421. doi: 10.1111/j.1365-294x.2012.05614.x

Hewitt, G. M. (1999). Post-glacial re-colonization of European biota. *Biol. J. Linnean Soc.* 68, 87–112. doi: 10.1111/j.1095-8312.1999.tb01160.x

Ho, J., Tumkaya, T., and Aryal, S. (2019). Moving beyond P values: data analysis with estimation graphics. *Nat. Methods* 16, 565–566. doi: 10.1038/s41592-019-0470-3

Holleley, C. E., and Geerts, P. G. (2009). Multiplex Manager 1.0: a crossplatform computer program that plans and optimizes multiplex PCR. *BioTechniques* 46, 511–517. doi: 10.2144/000113156

Jakobsson, M., and Rosenberg, N. A. (2007). CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23, 1801–1806. doi: 10.1093/bioinformatics/btm233

Kandemir, I., Kence, M., and Kence, A. (2005). Morphometric and electrophoretic variation in different honey bee (Apis mellifera L.) populations. *Turkish J. Veterinary Animal Sci.* 29, 885–890.

Kandemir, I., Kence, M., Sheppard, W. S., and Kence, A. (2006). Mitochondrial DNA variation in honey bee (Apis mellifera L.) populations from Turkey. *J. Apicultural Res.* 45, 33–38. doi: 10.3896/ibra.1.45.1.08

Kence, M., Farhoud, H. J., and Tunca, R. I. (2009). Morphometric and genetic variability of honey bee (Apis mellifera L.) populations from northern Iran. *J. Apicultural Res.* 48, 247–255. doi: 10.3896/ibra.1.48.4.04

Kükrer, M. (2013). *Genetic Diversity of Honey Bee Populations in Turkey Based on Microsatellite Markers: a Comparison Between Migratory Versus Stationary Apiaries and Isolated Regions Versus Regions Open to Migratory Beekeeping.* Master's thesis. Ankara: Middle East Technical University.

Kükrer, M., and Bilgin, C. (2020). Climate change prompts monitoring and systematic utilization of honey bee diversity in Turkey. *Bee Stud.* 12, 17–23. doi: 10.51458/bstd.2021.4

Kükrer, M., Kence, M., and Kence, A. (2020). Honey bee diversity is swayed by migratory beekeeping and trade despite conservation practices: genetic evidences for the impact of anthropogenic factors on population structure. *bioRxiv [preprint]* doi: 10.1101/154195v2

Langella, O. (2011). *Population Genetic Software: Individuals or Populations Distances Based on Allelic Frequencies, Phylogenetic Trees, File Conversions.* Massachusetts, MA: Bioinformatics Organization, Inc.

Letunic, I., and Bork, P. (2019). Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res.* 47, W256–W259. doi: 10.1093/nar/gkz239

Meffe, G. K. (1998). The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conserv. Biol.* 12, 8–17. doi: 10.1111/j.1523-1739.1998.97154.x

Morse, R. A. (1991). Honey bees forever. *Trends Ecol. Evol.* 6, 337–338.

Muñoz, I., and De la Rúa, P. (2020). Wide genetic diversity in Old World honey bees threaten by introgression. *Apidologie* 52, 200–217. doi: 10.1007/s13592-020-00810-0

Neumann, P., and Carreck, N. L. (2010). Honey bee colony losses. *J. Apicultural Res.* 49, 1–6. doi: 10.3896/ibra.1.49.1.01

Oldroyd, B. P. (2007). What's killing American honey bees? *PLoS Biology* 5:e168. doi: 10.1371/journal.pbio.0050168

Oleksa, A., Chybicki, I., Tofilski, A., and Burczyk, J. (2011). Nuclear and mitochondrial patterns of introgression into native dark bees (Apis mellifera mellifera) in Poland. *J. Apicultural Res.* 50, 116–129. doi: 10.3896/IBRA.1.50.2.03

Pinto, M. A., Henriques, D., Chávez-Galarza, J., Kryger, P., Garnery, L., van der Zee, R., et al. (2014). Genetic integrity of the Dark European honey bee (Apis mellifera mellifera) from protected populations: a genome-wide assessment using SNPs and mtDNA sequence data. *J. Apicultural Res.* 53, 269–278. doi: 10.3896/IBRA.1.53.2.08

Pritchard, J. K., Stephens, M., and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* 155, 945–959.

R Core Team (2013). *R: A Language and Environment for Statistical Computing.* Vienna: R Core Team.

Requier, F., Garnery, L., Kohl, P. L., Njovu, H. K., Pirk, C. W., Crewe, R. M., et al. (2019). The conservation of native honey bees is crucial. *Trends Ecol. Evol.* 34, 789–798. doi: 10.1016/j.tree.2019.04.008

Reynolds, J., Weir, B. S., and Clark Cockerham, C. (1983). Estimation of the coancestry coefficient: basis for a short-term genetic distance. *Genetics* 105, 767–779. doi: 10.1093/genetics/105.3.767

Rohlf, F. J., and Sokal, R. R. (1995). *Statistical Tables*. New York, NY: Macmillan.

Rosenberg, N. A. (2004). DISTRUCT: a program for the graphical display of population structure. *Mol. Ecol. Notes* 4, 137–138. doi: 10.1046/j.1471-8286.2003.00566.x

Ruttner, F. (1988). *Biogeography and Taxonomy of Honey Bees*. New York, NY: Springer V editor.

Shaibi, T., Lattorff, H. M. G., and Moritz, R. F. A. (2008). A microsatellite DNA toolkit for studying population structure in Apis mellifera. *Mol. Ecol. Resour.* 8, 1034–1036. doi: 10.1111/j.1755-0998.2008.02146.x

Solignac, M., Vautrin, D., Loiseau, A., Mougel, F., Baudry, E., Estoup, A., et al. (2003). Five hundred and fifty microsatellite markers for the study of the honeybee (Apis mellifera L.) genome. *Mol. Ecol. Notes* 3, 307–311. doi: 10.1046/j.1471-8286.2003.00436.x

Torchiano, M. (2016). Effsize—a package for efficient effect size computation. *Zenodo* doi: 10.5281/zenodo.196082

Tunca, R. I. (2009). *Determination and Comparison of Genetic Variation in Honey Bee (Apis Mellifera L.) Populations of Turkey by Random Amplified Polymorphic DNA and Microsatellite Analyses*. Ph.D. Thesis. Ankara: Middle East Technical University.

USDA NASS (2019). *Statistical Summary: Honey Bees. NASS Highlights*. Available online at: https://www.nass.usda.gov/Publications/Highlights/2019/2019_Honey_Bees_StatisticalSummary.pdf (accessed April 28, 2020).

Van Engelsdorp, D., Evans, J. D., Saegerman, C., Mullin, C., Haubruge, E., Nguyen, B. K., et al. (2009). Colony collapse disorder: a descriptive study. *PLoS One* 4:e6481. doi: 10.1371/journal.pone.0006481

Van Engelsdorp, D., and Meixner, M. D. (2010). A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. *J. Invertebrate Pathol.* 103, S80–S95.

Wallberg, A., Han, F., Wellhagen, G., Dahle, B., Kawata, M., Haddad, N., et al. (2014). A worldwide survey of genome sequence variation provides insight into the evolutionary history of the honeybee Apis mellifera. *Nat. Genet.* 46, 1081–1088. doi: 10.1038/ng.3077

Weinstock, G. M., Robinson, G. E., Gibbs, R., and Worely, K. C. (2006). Insights into social insects from the genome of the honey bee Apis mellifera. *Nature* 443, 931–949.

Whitfield, C. W., Behura, S. K., Berlocher, S. H., Clark, A. G., Johnston, J. S., Sheppard, W. S., et al. (2006). Thrice out of Africa: ancient and recent expansions of the honey bee, *Apis mellifera*. *Science* 314, 642–645.

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Morphological Changes in the Mandibles Accompany the Defensive Behavior of Indiana Mite Biting Honey Bees Against Varroa Destructor

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The honey bee (*Apis mellifera*) is the most important managed pollinator to sustainable agriculture and our ecosystem. Yet managed honey bee colonies in the United States experience 30–40% losses annually. Among the many biotic stressors, the parasitic mite *Varroa destructor* is considered one of the main causes of colony losses. Bees' mite-biting behavior has been selected as a *Varroa*-tolerant or *Varroa*-resistant trait in the state of Indiana for more than a decade. A survey of damaged mites from the bottom of a colony can be used as an extended phenotype to evaluate the mite-biting behavior of a colony. In this study, on average, 37% of mites sampled from the breeding stocks of 59 colonies of mite biters in Indiana were damaged or mutilated, which is significantly higher than the 19% of damaged mites found in commercial colonies in the southern United States. Indiana mite biter colonies had a higher proportion of damaged mites, although among the damaged mites, the number of missing legs was not significantly higher. In addition, the morphology of pollen-forager worker bee mandibles was compared by X-ray microcomputed tomography for six parameters in two populations, and a difference was detected in one parameter. Our results provide scientific evidence that potentially explains the defensive mechanism against *Varroa* mites: structural changes in the worker bee mandibles.

Keywords: honey bee, *Apis mellifera*, defensive behavior, *Varroa* resistance, bite, grooming, mandible, microCT

INTRODUCTION

Since 1987, when the ectoparasitic mite *Varroa destructor* was first introduced in the United States, *Varroa* infestations have become the primary contributors to honey bee (*Apis mellifera*) colony losses (Guzmán-Novoa et al., 2010; Le Conte et al., 2010; Nazzi and Le Conte, 2016). Mature *Varroa* females are 1.1 mm × 1.5 mm in size, and males are 0.8 mm × 0.7 mm (Häußermann et al., 2015). Most of the mite's life cycle happens inside the brood cells, including the egg, six-legged larva, protonymph, deutonymph, and adult developmental stages (Bailey, 1968; Genersch, 2010). *Varroa* mites can infest honey bee colonies and cause colony losses as they feed on the fat bodies of bee pupae and cause morphological and behavioral defects in bee development (Le Conte et al., 2010; Ramsey et al., 2019). Furthermore, the *Varroa* mite is an effective vector for the transmission of viruses within the honey bee colony (Di Prisco et al., 2011; Wilfert et al., 2016).

The European honey bee (*A. mellifera*) has developed a set of behavioral defenses against *Varroa* mites to keep the mite population low, such as grooming, biting, and performing hygienic behaviors (Ruttner and Hänel, 1992; Spivak, 1996; Arechavaleta-Velasco and Guzmán-Novoa, 2001; Guzman-Novoa et al., 2012; Tsuruda et al., 2012; Villa et al., 2017). The biting behavior of worker bees, which is also considered a type of grooming behavior, enables them to bite adult mites, and remove the mites from their bodies (Peng et al., 1987; Ruttner and Hänel, 1992; Pritchard, 2016). Colonies selected for mite-biting behavior by instrumental insemination or open mating with feral colonies potentially will have greater fitness over subsequent generations. Field reports show that Indiana mite biters from Purdue University, which have been selected over the past decade, have a higher mean proportion of damaged mites in the breeding population compared with unselected Italian queen bee colonies from California (Andino and Hunt, 2011; Hunt et al., 2016; Morfin et al., 2019). However, no report to date has compared changes in the mandibles as a potential mechanism for the mite-biting behavior.

Breeding mite-resistant bees is critical to maintaining sustainable apiculture for local pollination and food and crop productivity (Oddie et al., 2017). The modern beekeeping and breeding technique of instrumental insemination enables honey bee queens and colonies to be artificially selected (Meixner et al., 2010). Breeding efforts have been made on different continents and in different countries to select for mite-tolerant or mite-resistant traits (Spivak, 1996; Büchler et al., 2010; Rinderer et al., 2010). Mite-resistant bees assist beekeepers in managing the growing chemical miticide-resistance problems, and they will play a critical role in promoting sustainable agricultural practices (Kanga et al., 2016; Hamiduzzaman et al., 2017). In the past, research breeding efforts were focused on different bee-breeding stocks to improve the colony health (Büchler et al., 2010; Guarna et al., 2015, 2017), including in Russian bees (Rinderer et al., 2010), *Varroa*-Sensitive Hygienic bees (Danka et al., 2011; Villa et al., 2017), and Minnesota Hygienic bees (Spivak, 1996; Guarna et al., 2017).

Honey bee mandibles are considered the main mouthpart that worker bees use to bite or chew parasites, including mites and wax moths in the colony (Ruttner and Hänel, 1992). Our chemical analysis revealed that 2-heptanone is secreted from the mandibles and that it acts as an anesthetic on wax moth larva and *Varroa* mites (Papachristoforou et al., 2012). Micro-X-ray-computed tomography (microCT) is a technology that enables fast three-dimensional (3-D) scanning in satisfactory spatial resolution without complicated and lengthy sample preparation procedures. In the past, this technique has been used to study the brain anatomy and evolution of bees, ants, and other insects (Ribi et al., 2008; Li et al., 2011; Coty et al., 2014; Larabee et al., 2017). However, microCT has not previously been used to determine the shape of the honey bee mandible in fine detail.

In this study, we hypothesized that the Indiana mite-biter breeding stocks would have a higher level of mite-biting behavior than commercial bee colonies. Our goal was to characterize the bees' behavioral and morphological capacity for mite-biting behavior. The total number of mites, the percentage of damaged mites (as the parameter for determining mite-biting behavior), and the number of mite legs missing per colony were reported. In addition, the mechanism underlying the bees' mite-biting behavior was investigated by examining the shape of the mandibles in 3-D and comparing Indiana mite-biter colonies with commercial colonies from the southern United States (mainly the state of Georgia).

MATERIALS AND METHODS

Honey Bee Colonies

Fifteen colonies from five commercial sources were sampled from beekeepers who bought their package colonies in 2018, originally from the state of Georgia (from five different commercial providers). Mites were collected between September 19 and 26, 2018, from different areas in the state of Ohio (**Figure 1**, site a: one colony from Defiance, Ohio [Defianace1]; site b: 14 colonies from Bellbrook [NA1, NA2, NA3, NA5, and NA6], Beavercreek [PBJohn1 and PBJohn2], Cedarville [Dan4], and Wilberforce, Ohio [AB1, CSU23, CSU24, CSU32, CSU51, and CSU52]). In total, 59 colonies of Indiana mite-biter honey bees were sampled on July 3, August 6, September 28, October 10, October 17, and November 9, 2018, at Lafayette, Indiana (**Figure 1**, site c; colony numbers are listed in **Supplementary Table 1** of the **Supplementary Materials**). Fresh mite samples were collected over a 5-day period from each colony at Purdue University's main apiary. Some colonies were sampled twice. Seven colonies at the Wright Patterson Air Force Base (WPAFB), Huffman Prairie site housed seven virgin queens from Purdue stock colonies. All queens were open-mated with drones from feral colonies near WPAFB (within two miles). Mites from open-mated mite-biter colonies were collected from September 26 to 30, 2018.

For all the worker bee samples used for mandible scanning, pollen foragers returning to the hive entrance (either a commercial or Indiana mite-biter colony) were collected with an insect vacuum (No. 2820GA, BioQuip Products, United States). All the bees were then frozen on dry ice, transported back to the laboratory, and kept in an −80°C freezer. At least 10 foragers per colony were collected from each site. Indiana mite-biter bee samples were collected in June 2018, and commercial bee samples were collected in July 2018.

Varroa Mites

Three groups of mites were compared: (1) commercial colonies, (2) mite-biter colonies (from Indiana), and (3) open-mated mite-biter colonies (at WPAFB). **Figure 2** shows an example of a worker bee biting a *Varroa* mite on the top of a hive. Mite samples from Indiana mite-biter colonies were collected according to a previously described method (Andino and Hunt, 2011). For commercial bees and open-mated mite biters in Ohio, mite samples were collected as reported previously

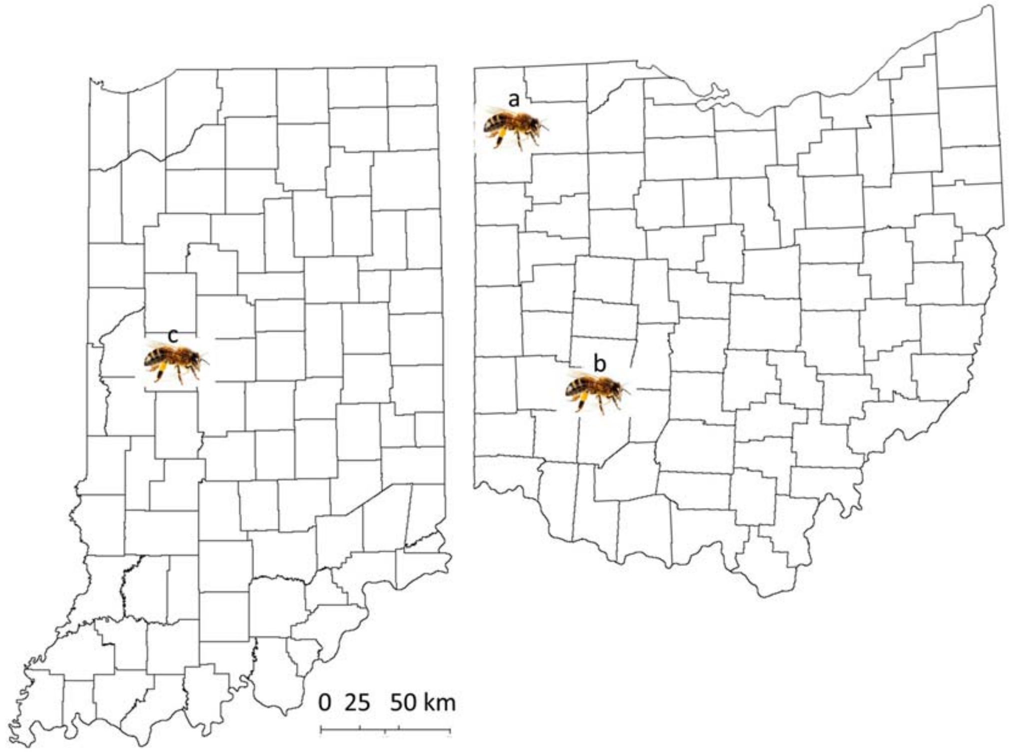

FIGURE 1 | Map of the locations of sample collection from the states of Ohio and Indiana. Site a: Defiance, Ohio; site b: Green County, Ohio, including the cities of Bellbrook, Beavercreek, Cedarville, and Wilberforce; site c: West Lafayette, Indiana, for Indiana mite-biter colonies.

(Andino and Hunt, 2011). A small paintbrush was used to remove mites from the bottom boards into a plastic disposable cup with a lid (1 oz. volume). Mite samples stayed in a −20°C freezer overnight. Each mite was carefully glued onto a glass microscope slide (25 mm × 75 mm × 1 mm, Globe Scientific Inc.) with a small paintbrush. Slides were examined under a light microscope (Zeiss STEMI 580) with a magnification of 50×. Colonies with 15 or more mites sampled within a 5-day period were included in the data analysis. Mites collected in Ohio were examined for any missing legs (from 1 to 8) as visible damage in the viewer (**Figure 3**). The total number of mites sampled per colony and the number of damaged mites were compared among the three groups (commercial, Indiana mite biters, and open-mated mite biters). The number of missing legs was compared between commercial colonies and open-mated mite-biter colonies. Immature mites and empty mite body shells were excluded.

MicroCT Scanning

From commercial colonies, five bees (five pairs of mandibles) were scanned from two colonies (three bees from colony PBJohn2 and two bees from colony Dan4). For the Indiana mite biters, nine bees from three colonies (three bees from colony 5, four bees from colony 15, and two bees from colony 41) were scanned. The scanning process was performed at the Center for Electron Microscopy and Analyses at Ohio State University (Columbus, Ohio) with a HeliScan microCT instrument (FEI Company, Thermo Fisher, United States) for 3-D imaging. A pair of mandibles was fixed to thin wooden posts ($r = 1$ mm, $h = 148$ mm) with superglue to fit in the HeliScan instrument. The scanning parameters were as follows: isotropic voxel size, 2.564 m per pixel; voltage, 60 or 80 kV; current, 80 or 46 μA; helical scan using space-filling trajectory reconstruction (Kingston et al., 2018) without any filter, 1,440 raw X-ray images. The software Avizo for FEI Systems (version 9.4, Thermo Fisher, United States) was used to quantify the measurements (height, length, width, small edge, long edge, and span of the spike area) for each scanned sample.

To compare the morphology of mandibles, six different parameters (**Figure 4**) were measured and compared between commercial colonies and mite-biter colonies. The height was measured from the top middle point to the base joint of the mandibular muscles. The long edge was the mandibular edge of the long side, similar to the blade on a pair of scissors. The short edge was the mandibular edge of the short side. The length was measured from the edge of the inner surface to the outer surface. The width was measured between the middle point of the long edge and the other side of the inner surface. The span of the spine area was the length of the sparse row of bristles or spines located along the inner side of the edge.

Data Analysis

The average number of mite samples per colony was compared among three groups, commercial bees, Indiana mite biters, and open-mated mite biters at WPAFB. If a colony was sampled

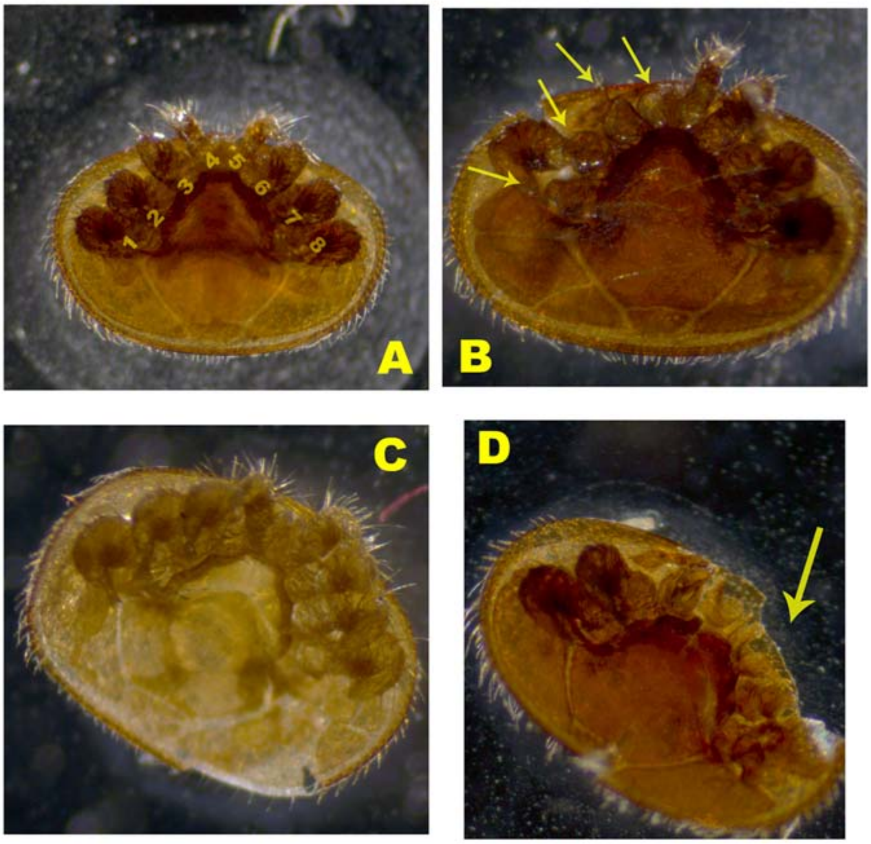

FIGURE 2 | Images of damaged *Varroa* mites from the sampled colonies. **(A)** Mature mite with no damage. Label numbers 1–8 indicate the eight legs of the *Varroa* mite. **(B)** Damaged mite with legs missing. **(C)** Young mature mite. **(D)** Mature mite with a missing body part. Arrows indicate the damaged legs or body part.

twice in the fall season, the average number of mites collected was used for statistical analysis. The ratio of damaged mites to the total number of sampled mites was transformed by using arcsine [square root (x)] for normal distribution. A one-way analysis of variance (ANOVA), with *post hoc* Tukey's honestly significant difference (HSD) test calculated for comparing multiple treatments, was used to determine the differences among means of the different populations. Dependent variables were the total number of mites, the mite-biting rate, the number of legs damaged, and the mandible parameters. The online tool Interactive Dotplot (Weissgerber et al., 2017) was used to generate all the box plots.

RESULTS

The mite-biting or grooming behavior referred to here involves a worker bee using its two forelegs and the two mandibles of its mouthpart to attack a *Varroa* mite in a colony. In addition to self-grooming, nest mates and groups of workers can actively remove adult female mites from worker bees and drop the damaged mites onto the bottom of the hive (Ruttner and Hänel, 1992). To characterize the damage to the mites, we categorized the observed mites into four different types: type A, mature adult female mites of a dark brown color with no damage and all eight legs present; type B, damaged adult female mites with legs missing; type C, young adult female mites of a pale color that were not counted as damaged adult mites; and type D, mites with body parts missing (**Figure 2**). Mite samples similar to type C were excluded because no damage was detected from worker bees' grooming or biting behavior.

To evaluate the total mite population, we collected all the *Varroa* mites that appeared on the bottom board of a colony from each commercial colony (15 colonies, N_{mite} = 886), each mite-biter colony (59 colonies, N_{mite} = 3,390), and each open-mated mite-biter colony (7 colonies, N_{mite} = 569). No significant difference was detected among the commercial colonies, mite-biter colonies, and open-mated mite-biter colonies (one-way ANOVA of three independent treatments), $F(2,80) = 1.20$, $p = 0.31$ (**Figure 3**).

To assess the bees' mite-biting or grooming behavior, we surveyed all the damaged mites collected from the bottom board of each colony for all three groups of colonies (commercial colonies, N_{mite} = 172, mite-biter colonies, N_{mite} = 1,201, open-mated mite-biter colonies, N_{mite} = 199). The means of the percentages of damaged mites per colony among the three colony types (commercial, mite biter, and open-mated mite biter) after transformation were 39.80, 60.22, and 60.14%. The one-way

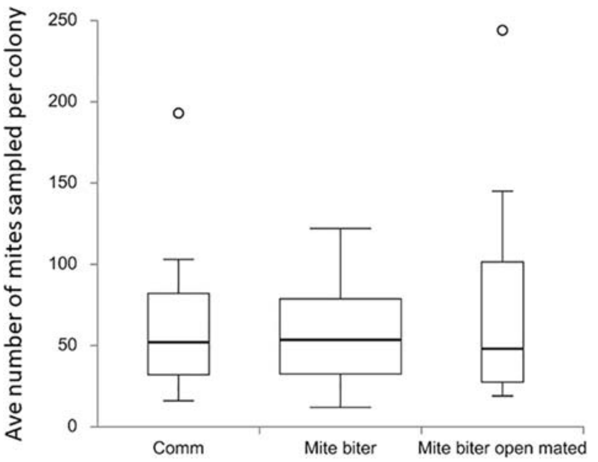

FIGURE 3 | Box plots of the average number of mites collected per colony among the three groups: commercial colonies (Comm, N_{colony} = 15), Indiana mite-biter colonies (mite biter, N_{colony} = 59), and open-mated mite-biter colonies (open-mated mite biter, N = 7). The open circles are outliers of the colonies, $F(2,80) = 1.20$, $p = 0.31$. The p-value corresponding to the F-statistic of the one-way ANOVA suggests that the three treatments were not significantly different.

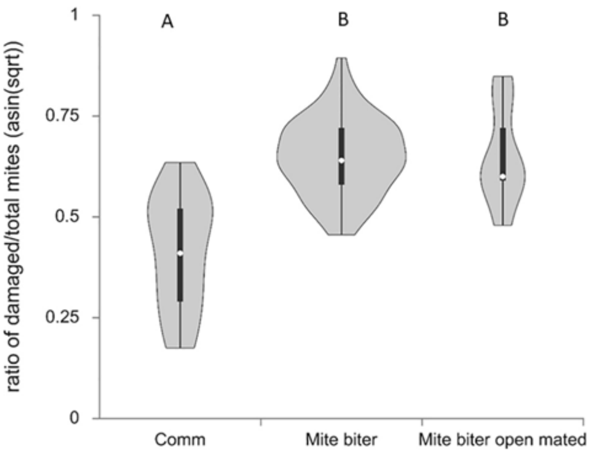

FIGURE 4 | Violin plots of the percentage of damaged mites identified in relation to total mites collected per colony among three groups, commercial colonies (Comm, N_{colony} = 15, N_{mite} = 172), Indiana mite-biter colonies (mite biter, N_{colony} = 59, N_{mite} = 1,201), and open-mated mite-biter colonies (open-mated mite biter, N_{colony} = 7, N_{mite} = 199). The one-way ANOVA suggested that one or more groups were significantly different, $F(2,80) = 28.86$, $p = 4.16e^{-10}$. The Tukey honestly significant difference test indicated that the levels of mite-biting behavior were not significantly different between the mite-biter and open-mated mite-biter colonies, but both were significantly higher than the commercial colonies, $Q = 10.63$ and 6.72, $p < 0.01$.

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To further evaluate the bees' damage to mites and the potential difference between commercial colonies and open-mated mite-biter colonies, we counted the number of legs missing from each damaged mite. This result showed no difference in the average number of legs missing per mite between these two groups, $Q = 2.43$, $p > 0.05$ (**Figure 6**).

To compare the morphology of mandibles between commercial colonies and mite-biter colonies, we measured six different parameters from the microCT data: the length, width, height, long edge, short edge, and span of the spine area (**Figure 4**). The ANOVA between these two groups showed that the long edge of mandibles in the mite-biter colonies were significantly shorter than those in commercial colonies, $F = 5.78$, $p = 0.03$ (**Figure 7**). We found no significant difference between the two groups in the other five parameters (**Figure 7**), but in the length, height, short edge, and span of the spine area, we noticed a consistent trend of smaller values in the mite-biter colonies.

DISCUSSION

We investigated the *Varroa* mite population and the differences in mite-biting behavior among commercial colonies, mite-biter colonies, and open-mated mite-biter colonies in

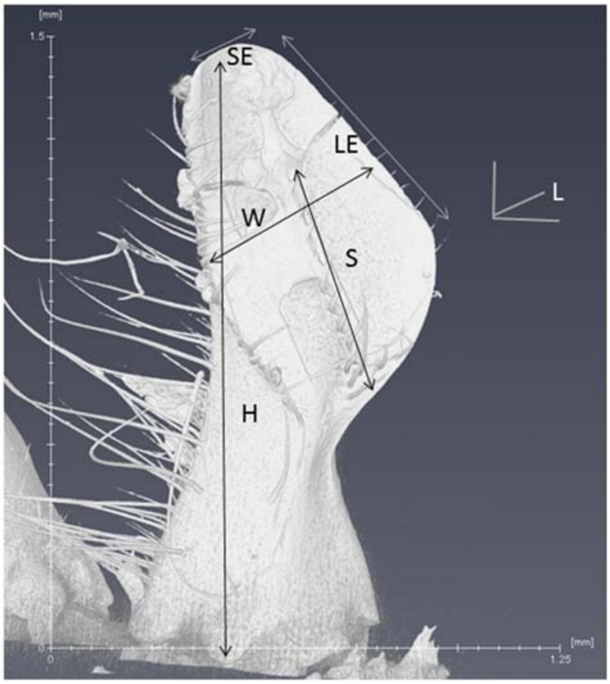

FIGURE 5 | Box plots of the average number of legs missing per mite per colony among two groups, commercial colonies (Comm, N_{colony} = 15) and open-mated mite-biter colonies (open-mated mite biter, N_{colony} = 7). The open circles indicate outliers. The results showed no difference in the average number of legs missing per mite between the two groups, $Q = 2.43$, $p > 0.05$.

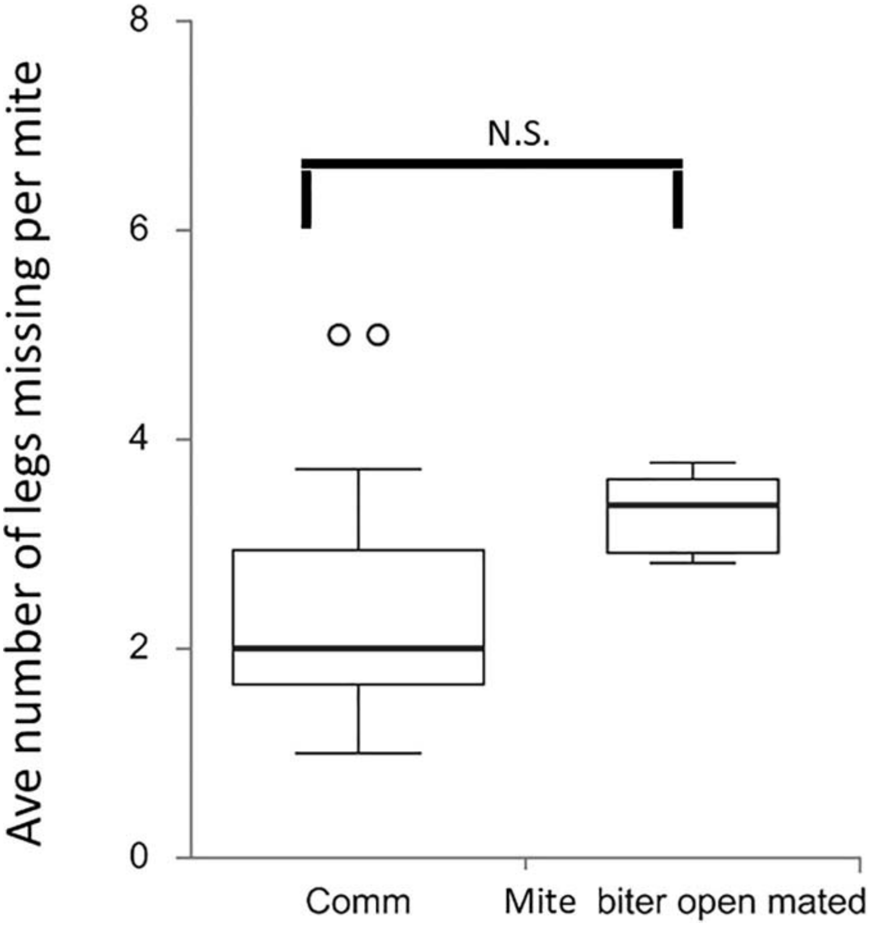

FIGURE 6 | Six parameters of the scanned mandible are listed on the microCT image. L, length; W, width; H, height; SE, short edge; LE, long edge; S, span of the spine area.

the United States. In addition, we evaluated differences in the shape of bee mandibles between mite-biter stock colonies and commercial colonies. Bees in the mite-biter colonies displayed a higher level of mite-biting behavior than did those in the commercial colonies. The difference in the long edge of their mandibles may explain the physical mechanism by which their mandibles are able to mutilate mites.

Previous research on grooming behavior and damaged mites in *Apis* has shown that grooming behavior is a selected trait in naturally mite-resistant colonies (Peng et al., 1987; Boecking and Ritter, 1993; Fries et al., 1996; Arechavaleta-Velasco and Guzmán-Novoa, 2001; Russo et al., 2020). The mite-biting or grooming behavior of honey bees, as a defensive behavior against parasitic *Varroa* mites, can be used as a parameter to select for *Varroa* mite resistance in honey bee stocks (Spivak, 1996; Rinderer et al., 2010; Hunt et al., 2016; Pritchard, 2016; Morfin et al., 2019).

Our present comparison between commercial and mite-biter bees supports the value of selecting for mite-biting behavior in *A. mellifera*. With freshly damaged mites as our evidence, we provided a strong argument that workers of *A. mellifera* are able to amputate the legs of *Varroa* mites, as described by Ruttner and Hänel (1992). Collecting damaged mites from the bottom board of each colony within a 5-day time frame ensured that the observed damage on the mites was fresh. It is possible for beekeepers to record the proportion of damaged mites from the bottom boards of colonies and make a collaborative regional effort to select for the mite-biting trait in their region (Bienefeld et al., 1999; Hunt et al., 2016).

As a defensive response, honey bees use biting or grooming behavior to decrease the infestation of a mite population in the colony. Worker bees are known to use their mandibles to mutilate or damage *Varroa* mites, as reported previously (Ruttner and Hänel, 1992). Further studies have shown that certain chemicals, such as 2-heptanone, can be released from workers' mandibles during a bite to anesthetize parasites in honey bee colonies, including *Varroa* mites and wax moths (Papachristoforou et al., 2012). Although bees' mite-biting behavior has been reported, the underlying mechanism for this behavior has not been reported. Our results provide empirical evidence for changes in the structure of mandibles, such as the length of the long edge, which could be the mechanism

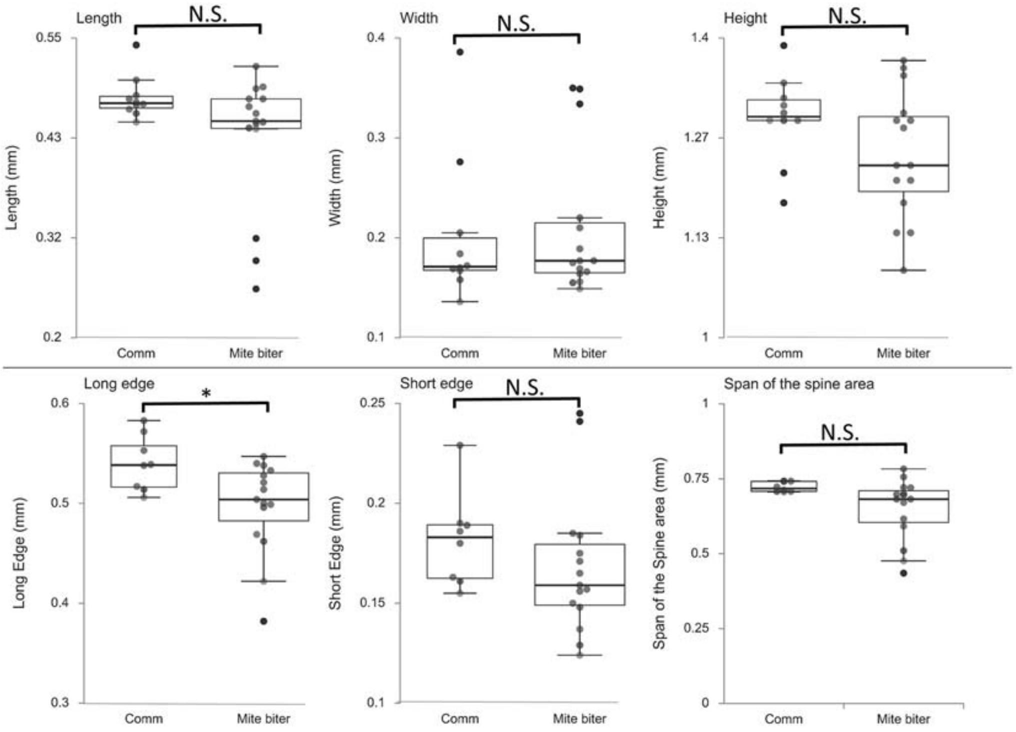

FIGURE 7 | Box plots of the six parameters of mandible morphology between two groups, commercial colonies (Comm, $N_{\text{mandible}} = 10$) and mite-biter colonies (mite biter, $N_{\text{mandible}} = 15$). Each filled circle represents a data point. Results of the one-way ANOVA suggest that the two treatments were not significantly different in length, width, height, short edge, or span of the spine area, but they were significantly different in the long edge, $F(1,21) = 5.78$, $p < 0.05$.

underlying the biting behavior. In addition, our data showed that mite biters are under selection, which may lead to such structural changes toward mite resistance. Eastern honey bees (*A. cerana*) are the original hosts of *Varroa* mites, and in Asia, they have now evolved to be *Varroa* mite resistant. Their body size is also slightly smaller than that of *A. mellifera* (Peng et al., 1987; Yue et al., 2018). It is not clear if the change of body size is related to the evolution of biting behavior.

Our data indicate a clear trend that a behavioral adaptation is evolving in mite biters to defend against the parasitic mite *V. destructor*. These bees engaged in greater mite-biting behavior, perhaps because of their greater sensitivity to the mites, given the similarly high numbers of mite populations in all three colonies. Morfin et al. (2019) previously showed that the mite population was reduced in mite-biter colonies compared with colonies unselected for mite biting. However, our comparison of the total numbers of mites showed no significant difference among the commercial, mite-biter, and open-mated mite-biter colonies. This may be because the colonies we tested were in different geographic locations and had different management histories. Another possible mechanism for behavioral adaptation, via genetic changes such as the gene *AmNrx-1*, has been reported by Morfin et al. (2019).

The worker bees' ability to detect mites may be based on their olfactory ability, considering that mite-biting behavior happens in dark hives most of the time. The mite-biter stocks may show a greater capability of detecting and recognizing mites as pests than do commercial colonies not selected for mite biting. Gradual changes may be taking place in the relationship between *A. mellifera* and *Varroa* mites. One potential change may be the biting mechanism of mite biters, one that Asian honey bees now display, as reported by Peng et al. (1987). Compared with the low frequency of mite removal and the limited success in clearing mites of bees in commercially sourced colonies, bees in mite-biter colonies exhibited an improvement in these abilities.

We identified the long edge of the mandible in bees from mite biter colonies as being shorter than that of bees from commercial colonies. The long edge is like a sharp knife that can be used to cut off the hind legs of *Varroa* mites. The pair of mandibles can act as a tool with double edges on the basal half of the rim. The mandibles adhere to the surface of mandibular muscles on the head. Potential differences of muscles may be related to the difference of biting ability among difenert populations. These structures may explain why the change in the long edge affects the ability of workers to bite the mites. Even though other measurements did not show a significant difference, similar trends were observed in the short edge, height, and length.

Although evidence exists for variation in the mite-biting or grooming behavior of different genotypes (Guzman-Novoa et al., 2012), more research is needed on the genetic architecture and pattern of inheritance of this behavior for honey bee breeding and selection. With the rapid development of new sequencing technologies and genome editing tools, genome-wide marker-assisted selection may be applied in the future for honey bee breeding.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

HL-B conceived of and designed the experiments. JS, XC, KG, and HL-B collected samples and data. JS and HL-B performed the data analysis and wrote the manuscript. All authors approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.638308/full#supplementary-material

REFERENCES

Andino, G. K., and Hunt, G. J. (2011). A scientific note on a new assay to measure honeybee mite-grooming behavior. *Apidologie* 42, 481–484. doi: 10.1007/s13592-011-0004-1

Arechavaleta-Velasco, M. E., and Guzmán-Novoa, E. (2001). Relative effect of four characteristics that restrain the population growth of the mite *Varroa destructor* in honey bee (*Apis mellifera*) colonies. *Apidologie* 32, 157–174. doi: 10.1051/apido:2001121

Bailey, L. (1968). Honey bee pathology. *Annu. Rev. Entomol.* 13, 191–212. doi: 10.1146/annurev.en.13.010168.001203

Bienefeld, K., Zautke, F., Pronin, D., and Mazeed, A. (1999). Recording the proportion of damaged *Varroa jacobsoni* Oud. in the debris of honey bee colonies (*Apis mellifera*). *Apidologie* 30, 249–256. doi: 10.1051/apido:19990401

Boecking, O., and Ritter, W. (1993). Grooming and removal behaviour of *Apis mellifera intermissa* in Tunisia against *Varroa jacobsoni*. *J. Apic. Res.* 32, 127–134. doi: 10.1080/00218839.1993.11101297

Büchler, R., Berg, S., and Le Conte, Y. (2010). Breeding for resistance to *Varroa destructor* in Europe. *Apidologie* 41, 393–408. doi: 10.1051/apido/2010011

Coty, D., Aria, C., Garrouste, R., Wils, P., Legendre, F., and Nel, A. (2014). The first ant–termite syninclusion in amber with CT-scan analysis of taphonomy. *PLoS One* 9:e104410. doi: 10.1371/journal.pone.0104410

Danka, R. G., Harris, J. W., and Villa, J. D. (2011). Expression of *Varroa* sensitive hygiene (VSH) in commercial VSH honey bees (Hymenoptera: Apidae). *J. Econ. Entomol.* 104, 745–749. doi: 10.1603/ec10401

Di Prisco, G., Pennacchio, F., Caprio, E., Boncristiani, H. F. Jr., Evans, J. D., and Chen, Y. (2011). Varroa destructor is an effective vector of Israeli acute paralysis virus in the honeybee, *Apis mellifera*. *J. Gen. Virol.* 92, 151–155. doi: 10.1099/vir.0.023853-0

Fries, I., Wei, H. Z., Shi, W., and Chen, S. J. (1996). Grooming behavior and damaged mites (*Varroa jacobsoni*) in *Apis cerana cerana* and *Apis mellifera ligustica*. *Apidologie* 27, 3–11. doi: 10.1051/apido:19960101

Genersch, E. (2010). Honey bee pathology: current threats to honey bees and beekeeping. *Appl. Microbiol. Biotechnol.* 87, 87–97. doi: 10.1007/s00253-010-2573-8

Guarna, M. M., Hoover, S. E., Huxter, E., Higo, H., Moon, K.-M., Domanski, D., et al. (2017). Peptide biomarkers used for the selective breeding of a complex polygenic trait in honey bees. *Sci. Rep.* 7:8381. doi: 10.1038/s41598-017-08464-2

Guarna, M. M., Melathopoulos, A. P., Huxter, E., Iovinella, I., Parker, R., Stoynov, N., et al. (2015). A search for protein biomarkers links olfactory signal transduction to social immunity. *BMC Genomics* 16:63. doi: 10.1186/s12864-014-1193-6

Guzmán-Novoa, E., Eccles, L., Calvete, Y., McGowan, J., Kelly, P. G., and Correa-Benítez, A. (2010). *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. *Apidologie* 41, 443–450. doi: 10.1051/apido/2009076

Guzman-Novoa, E., Emsen, B., Unger, P., Espinosa-Montaño, L. G., and Petukhova, T. (2012). Genotypic variability and relationships between mite infestation levels, mite damage, grooming intensity, and removal of *Varroa destructor* mites in selected strains of worker honey bees (*Apis mellifera* L.). *J. Invertebr. Pathol.* 110, 314–320. doi: 10.1016/j.jip.2012.03.020

Hamiduzzaman, M. M., Emsen, B., Hunt, G. J., Subramanyam, S., Williams, C. E., Tsuruda, J. M., et al. (2017). Differential gene expression associated with honey bee grooming behavior in response to *Varroa* mites. *Behav. Genet.* 47, 335–344. doi: 10.1007/s10519-017-9834-6

Häußermann, C. K., Ziegelmann, B., Bergmann, P., and Rosenkranz, P. (2015). Male mites (*Varroa destructor*) perceive the female sex pheromone with the sensory pit organ on the front leg tarsi. *Apidologie* 46, 771–778. doi: 10.1007/s13592-015-0367-9

Hunt, G., Given, J. K., Tsuruda, J. M., and Andino, G. K. (2016). Breeding mite-biting bees to control *Varroa*. *Bee Cult.* 8, 41–47.

Kanga, L. H. B., Marshall, K., and Legaspi, J. C. (2016). Mechanisms of insecticide resistance in field populations of the *Varroa* mite (Acari: Mesostigmata: Varroidae) in Florida. *Fla. Entomol.* 99, 324–326. doi: 10.1653/024.099.0231

Kingston, A. M., Myers, G. R., Latham, S. J., Recur, B., Li, H., and Sheppard, A. P. (2018). Space-filling X-ray source trajectories for efficient scanning in large-angle cone-beam computed tomography. *IEEE Trans. Comput. Imaging* 4, 447–458. doi: 10.1109/TCI.2018.2841202

Larabee, F. J., Gronenberg, W., and Suarez, A. V. (2017). Performance, morphology and control of power-amplified mandibles in the trap-jaw ant *Myrmoteras* (Hymenoptera: Formicidae). *J. Exp. Biol.* 220, 3062–3071. doi: 10.1242/jeb.156513

Le Conte, Y., Ellis, M., and Ritter, W. (2010). *Varroa* mites and honey bee health: can *Varroa* explain part of the colony losses? *Apidologie* 41, 353–363. doi: 10.1051/apido/2010017

Li, D., Zhang, K., Zhu, P., Wu, Z., and Zhou, H. (2011). 3D configuration of mandibles and controlling muscles in rove beetles based on micro-CT technique. *Anal. Bioanal. Chem.* 401, 817–825. doi: 10.1007/s00216-011-5088-y

Meixner, M. D., Costa, C., Kryger, P., Hatjina, F., Bouga, M., Ivanova, E., et al. (2010). Conserving diversity and vitality for honey bee breeding. *J. Apic. Res.* 49, 85–92. doi: 10.3896/ibra.1.49.1.12

Morfin, N., Given, K., Evans, M., Guzman-Novoa, E., and Hunt, G. J. (2019). Grooming behavior and gene expression of the Indiana "mite-biter" honey bee stock. *Apidologie* 51, 267–275. doi: 10.1007/s13592-019-00710-y

Nazzi, F., and Le Conte, Y. (2016). Ecology of *Varroa destructor*, the major ectoparasite of the western honey bee, *Apis mellifera*. *Annu. Rev. Entomol.* 61, 417–432. doi: 10.1146/annurev-ento-010715-023731

Oddie, M., Dahle, B., and Neumann, P. (2017). Norwegian honey bees surviving *Varroa destructor* mite infestations by means of natural selection. *PeerJ* 5:e3956. doi: 10.7717/peerj.3956

Papachristoforou, A., Kagiava, A., Papaefthimiou, C., Termentzi, A., Fokialakis, N., Skaltsounis, A.-L., et al. (2012). The bite of the honeybee: 2-heptanone secreted from honeybee mandibles during a bite acts as a local anaesthetic in insects and mammals. *PLoS One* 7:e47432. doi: 10.1371/journal.pone.0047432

Peng, Y. S., Fang, Y. Z., Xu, S. Y., and Ge, L. S. (1987). The resistance mechanism of the Asian honey-bee, *Apis-cerana* Fabr., to an ectoparasitic mite, *Varroa jacobsoni* Oudemans. *J. Invert. Pathol.* 49, 54–60. doi: 10.1016/0022-2011(87)90125-x

Pritchard, D. J. (2016). Grooming by honey bees as a component of *Varroa* resistant behavior. *J. Apic. Res.* 55, 38–48. doi: 10.1080/00218839.2016.1196016

Ramsey, S. D., Ochoa, R., Bauchan, G., Gulbronson, C., Mowery, J. D., Cohen, A., et al. (2019). *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. *Proc. Natl. Acad. Sci. U.S.A.* 116, 1792–1801. doi: 10.1073/pnas.1818371116

Ribi, W., Senden, T. J., Sakellariou, A., Limaye, A., and Zhang, S. (2008). Imaging honey bee brain anatomy with micro-X-ray-computed tomography. *J. Neurosci. Methods* 171, 93–97. doi: 10.1016/j.jneumeth.2008.02.010

Rinderer, T. E., Harris, J. W., Hunt, G. J., and De Guzman, L. I. (2010). Breeding for resistance to *Varroa destructor* in North America. *Apidologie* 41, 409–424. doi: 10.1051/apido/2010015

Russo, R. M., Liendo, M. C., Landi, L., Pietronave, H., Merke, J., Fain, H., et al. (2020). Grooming behavior in naturally *Varroa*-resistant *Apis mellifera* colonies from north-central Argentina. *Front. Ecol. Evol.* 8:361. doi: 10.3389/fevo.2020.590281

Ruttner, F., and Hänel, H. (1992). Active defense against *Varroa* mites in a Carniolan strain of honeybee (*Apis mellifera carnica* Pollmann). *Apidologie* 23, 173–187. doi: 10.1051/apido:19920210

Spivak, M. (1996). Honey bee hygienic behavior and defense against *Varroa jacobsoni*. *Apidologie* 27, 245–260. doi: 10.1051/apido:19960407

Tsuruda, J. M., Harris, J. W., Bourgeois, L., Danka, R. G., and Hunt, G. J. (2012). High-resolution linkage analyses to identify genes that influence *Varroa* sensitive hygiene behavior in honey bees. *PLoS One* 7:e48276. doi: 10.1371/journal.pone.0048276

Villa, J. D., Danka, R. G., and Harris, J. (2017). Repeatability of measurements of removal of mite-infested brood to assess *Varroa* sensitive hygiene. *J. Apic. Res.* 56, 631–634. doi: 10.1080/00218839.2017.1369707

Weissgerber, T. L., Savic, M., Winham, S. J., Stanisavljevic, D., Garovic, V. D., and Milic, N. M. (2017). Data visualization, bar naked: a free tool for creating interactive graphics. *J. Biol. Chem.* 292, 20592–20598. doi: 10.1074/jbc.RA117.000147

Wilfert, L., Long, G., Leggett, H., Schmid-Hempel, P., Butlin, R., Martin, S., et al. (2016). Deformed wing virus is a recent global epidemic in honeybees driven by *Varroa* mites. *Science* 351, 594–597. doi: 10.1126/science.aac9976

Yue, M., Luo, S., Liu, J., and Wu, J. (2018). *Apis cerana* is less sensitive to most neonicotinoids, despite of their smaller body mass. *J. Econ. Entomol.* 111, 39–42. doi: 10.1093/jee/tox342

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Defensive Behavior and Morphometric Variation in *Apis mellifera* Colonies From Two Different Agro-Ecological Zones of North-Western Argentina

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European lineages of *Apis mellifera* were first introduced into America for beekeeping purposes. A subsequent introduction and accidental release of *A. m. scutellata* resulted in hybridization events that gave rise to Africanized populations that rapidly spread throughout the continent. In Argentina, Africanized honey bees (AHBs) have been mostly detected in northern regions of the territory, and represent a valuable genetic resource for the selection of stocks with advantageous characteristics for beekeeping. The objective of the present study was to profile honey bee colonies of wild origin with potential beneficial traits for apiculture using morphological, molecular and behavioral traits. Honey bee colonies chosen for evaluation were located in two different agro-ecological regions in north-western Argentina (Tucumán province): The Chaco Depressed Plain (Leales apiary) and the Piedmont (Famaillá apiary). Each apiary was surveyed three times during the 2017–2018 season (mid-season, wintertime, and early spring) for: brood population, phoretic *Varroa* level and defensive behavior (run, fly, sting, and hang). At the midpoint of the beekeeping season colonies were also characterized by morphometry (45 variables) and mitochondrial haplotypes (COI–COII intergenic region). Apiaries studied showed similar patterns throughout the beekeeping season, for most of the characteristics monitored. However, significant variation in defensive behavior parameters was found between apiaries at the different times of evaluation. Twelve of 45 morphometric variables also showed significant differences between apiaries. The mitochondrial haplotype analysis revealed a high representation of African A4 and A1 haplotypes (91%) in both apiaries. Haplotype variation was associated with morphometric and behavioral traits. Multivariate analyses [principal component analysis (PCA) and principal coordinate analysis (PCoA)] including morphometric and behavior variables explained 65.3% (PCA) and 48.1% (PCoA) of the variability observed between colonies in the first two components. Several morphometric parameters and "fly" behavior were mainly associated with the separation of the colonies. The results from this study point to a possible association between morphometric and behavioral variation and the adaptation of honey bee colonies to differential agro-ecological conditions. We discuss how the detected variation between apiaries can be used for the selection and preservation of honey bee ecotypes in regional breeding programs.

Keywords: defensiveness, honey bees, environment, mitochondrial haplotype, morphometry

INTRODUCTION

The western honey bee, *Apis mellifera* (Hymenoptera: Apidae), plays a crucial role in crop pollination and is considered the most important honey producer worldwide (Navajas et al., 2008; Van Engelsdorp and Meixner, 2010; Guzmán-Novoa et al., 2011; Iqbal et al., 2019). This species has shown great adaptive potential, as it has established in diverse environments (Le Conte and Navajas, 2008; Meixner et al., 2013). In their natural range (across Europe, the Middle East, and Africa), more than 26 morphologically and geographically distinct *A. mellifera* subspecies have been described (Ruttner, 1988).

Using morphology and genetic analysis, *A. mellifera* subspecies have been assigned to four main evolutionary branches or lineages (A, C, M, and O) (Ruttner, 1988; Franck et al., 2000; Whitfield et al., 2006). *A. mellifera ligustica*, *A. m. mellifera* and *A. m. carnica* subspecies (assigned to C and M European lineages) were established in the Americas for apicultural practices in the early 19^{th} century (Bierzychudek, 1979; Salizzi, 2014). In 1956, scientists introduced the African subspecies *A. m. scutellata* (A lineage) into Brazil for the purpose of improving the genetics of honey bees established in tropical climates. An accidental release of these honey bees of African origin and their uncontrolled dispersion, along with the concomitant hybridization with European genotypes, led to a process of "Africanization" of the then resident *A. mellifera* (Kerr and Nielsen, 1967; Kent, 1988). Africanized populations spread throughout America, increasing the genetic diversity of local resources or ecotypes (Buco et al., 1986; Sheppard et al., 1991; Guzmán-Novoa et al., 2011).

Africanized honey bee (AHB) colonies have retained some characteristic traits from their African ancestors, such as high defensive behavior, tendency to swarm and abscond, tolerance to the mite *Varroa destructor*, adaptation to subtropical and tropical climates, and a smaller body size (Breed et al., 2004; Schneider et al., 2004; Francoy et al., 2008; Guzmán-Novoa et al., 2011; Rivera-Marchard et al., 2012). Conversely, European honey bees have been associated with low defensiveness, swarming and absconding, along with high honey production and adaptation to temperate climate (De Grandi-Hoffman et al., 1998; Breed et al., 2004; Hunt, 2007; Medina-Flores et al., 2014).

As a result of hybridization processes, behavioral and genetic variation has been observed in Africanized populations from America. This variation is also attributed to the adaptation of honey bees to different geographic and environmental conditions (Southwick and Moritz, 1987; Guzmán-Novoa and Page, 1999; Breed et al., 2004). Several investigations have characterized Africanized populations established throughout America by means of morphology (Buco et al., 1986; Francoy et al., 2008), behavior (Alaux et al., 2009; Guzmán-Novoa et al., 2011), and genetics (Collet et al., 2006, 2009; Whitfield et al., 2006; Acevedo-Gonzalez et al., 2019), including integrative approaches (Rivera-Marchard et al., 2012). In Argentina, a high genetic variability has been detected in commercial and feral colonies (Agra et al., 2018; Calfee et al., 2020). Furthermore, the latter listed authors, confirmed results previously obtained by Sheppard et al. (1999), regarding the presence of populations derived from different lineages: *A. mellifera scutellata* (A4 and A1), *A. mellifera intermissa*, and *A. m iberiensis* distributed mainly in the northern region of the country. The presence of the A1 haplotype suggests that a second influx of honey bees from Africa to South America occurred, and it is considered as another source of Africanization of the Argentinian honey bee populations (Sheppard et al., 1999; Agra et al., 2018).

The honey bee breeding program of Argentina (MeGA, PROAPI) has focused on the selection, preservation and augmentation of honey bee stocks for beekeeping in different agro-ecological regions of the country (Palacio et al., 2000; Bedescarrabure, 2011). The selection criterion includes hygienic behavior, tolerance to brood diseases, low defensiveness, and high productivity. In this framework, the detection and characterization of wild-origin honey bee colonies with desirable characteristics already adapted to the diverse environmental conditions of Argentina is of fundamental importance to the development of sustainable apiculture at the regional level.

In the present study, we describe colony parameters (strength, brood population and phoretic *Varroa* levels), in conjunction with morphometric, defensive behavior, and genetic characteristics of two apiaries with wild honey bee colonies located in different agro-ecological regions of north-western Argentina (Tucumán province). Colonies were inspected three times (middle-productive season, wintertime, and early spring) during the 2017–2018 season. We discuss our results by considering the potential environmental factors involved in shaping the differences observed between the apiaries evaluated in this study. The results obtained provide a first screening of useful tools for the selection of honey bees with the desirable characteristics of tolerance to colony diseases and low defensiveness while also adapted to a subtropical climate. In addition, this study provides the basis for the need to preserve honey bee Africanized genetic resources in Argentina given the current threat of unpredictable climate change.

MATERIALS AND METHODS

Honey Bee Colonies

Apis mellifera colonies from the Leales and Famaillá apiaries were surveyed in the present study. Each apiary was composed of feral colonies that originated from the natural occupation of empty hives by honey bees from surrounding areas. There are no commercial apiaries within a radius of 5 km, where the empty hives were located.

Leales apiary (hereafter named "LE") is located at Instituto de Investigación Animal del Chaco Semiárido (IIACS-INTA), Santa Rosa de Leales, Tucumán province (27°8′15″ S 65°15′42″ W), a region characterized by a saline depressed plain (Zuccardi and Fadda, 1985; Collantes and Busnelli, 2014; **Figure 1**). In this region, agricultural activity is focused predominantly on the cultivation of sugar cane (258,851 ha), followed by the production of soybean and corn (Benedetti et al., 2019). Famaillá apiary (hereafter named "FAM") is situated at Estación Experimental Agropecuaria (EEA) Famaillá (INTA), Famaillá, Tucumán province (27°3′14.87″ S 65°24′11.84″ W), in a non-saline depressed plain (Zuccardi and Fadda, 1985) or piedmont (Collantes and Busnelli, 2014) (**Figure 1**). The predominant agricultural product in this region is citrus fruits (49,128 ha), followed by the cultivation of sugar cane (Benedetti et al., 2019).

Of the 11 feral colonies selected for the present survey, seven were from FAM and four from LE (**Supplementary Table 1**). Colonies were selected according to the following criteria: (1) colony strength, more than seven frames covered by honey bees during beekeeping mid-season (hereafter named mid-season), category = 1 based on the "Beekeeping Manual for Subtropical Environments" (Dini and Bedescarrabure, 2011); and (2) colonies naturally-tolerant to the mite *V. destructor* as determined by survival for one season without mite control treatment. During the survey, the colonies from both apiaries did not receive any acaricide treatment, and were managed using the same protocol of good beekeeping practices (Dini and Bedescarrabure, 2011; Unger et al., 2013).

The inspections of honey bee colonies were performed at three different times during the 2017–2018 season: mid-season (December 2017), wintertime (July 2018), and early spring (September 2018). During the colony survey the following parameters were monitored: colony strength, honey bee brood population, phoretic *Varroa*, and defensive behavior. At mid-season, 20 nurse bees were randomly taken from the center of each colony and preserved in ethanol 96% (v/v) for further molecular and morphometric analyses (described below).

Colony Status Measurements

Colony strength was assessed by visual inspection of the top of the hive following the procedure described by Unger et al. (2013) and Figini et al. (2017). Three different categories used were as follows: category 1 (at least seven frames covered by honey bees); category 2 (five to seven frames covered by bees); and category 3 (fewer than five frames covered by bees).

The brood population was estimated for each colony as the total area of combs covered by brood according to De Grandi-Hoffman et al. (1998). Briefly, (1) hives were opened and frames sequentially removed; (2) a panel subdivided into quadrants of equal size was superimposed on each frame and an estimate was made of the area covered by brood; and (3) a total count was made of the number of frames fully occupied by brood.

The evaluation of phoretic *Varroa* was done through the "jar test" according to De Jong et al. (1982) and Dietemann et al. (2013). Briefly, a sample of about 250-300 bees from at least two brood combs (both sides) were swept into a bottle containing 70% v/v ethanol. The bottle was energetically shaken and ethanol filtered through a special mesh and white cloth to separate *Varroa* mites from the adult worker bees. Once separated, both groups were counted and the percentage of mites present in the sample was calculated based on the total number of bees (% phoretic *Varroa* = mites/bees × 100) (De Jong et al., 1982; Dietemann et al., 2013).

Genetic Characterization of the Colonies

DNA was extracted from the thorax of one worker from each colony per apiary following the methods outlined in Sheppard et al. (1991). A total of 11 individuals were analyzed. A partial region of the mitochondrial COI-COII intergenic region was amplified using 25μl PCR reactions using primers and conditions described by Hall and Smith (1991) and Lobo Segura (2000) with some modifications according to Agra et al. (2018). The 25 μl PCR reaction mix consisted of 1 μM of each primer, 0.5 mM of PCR nucleotide mix (Genbiotech, Buenos Aires, Argentina), 1.5 mM $MgCl_2$ (Inbio Highway, Tandil, Argentina), 19 μl reaction buffer (InbioHighway), 1 U Taq Polymerase (InbioHighway), and 5 μl of DNA template. The PCR amplifications were conducted in a MJ PTC-100 thermal cycler (GMI, Ramsey, MN, United States) with a cycling protocol that consisted of an initial denaturation step of 2 min at 94°C followed by 40 cycles of 30s at 94°C, 30s at 55°C, and 1 min at 72°C, with a final extension step of 2 min at 72°C. To obtain restriction fragments a10-μl aliquot of each PCR product was digested with *Hin*fI (Promega, Madison, MN, United States) following manufacturer recommendations. Restriction fragments were separated on 4% (w/v) agarose gels, stained with GelRed, and photographed under UV light following Agra et al. (2018).

Morphometric Measurements

Morphometric data of honey bee workers were obtained from the right hind leg, proboscis, and right fore and hind wings. To perform the measurements, the aforementioned body parts were mounted on glass slides (one individual per slide; 10 individuals per colony and apiary), for a total of 110 preparations. The preparations were then photographed, digitized, and morphological characters measured and analyzed.

Twelve traditional morphometry parameters previously described for honey bees (Andere et al., 2008) were measured following the protocol developed by Padilla et al. (2001). Variables considered from each body part were as follows: right hind leg (metatarsal width: LegL4 as shown in **Figure 2A**); proboscis length (ProbL; **Figure 2B**); right fore wing [five angles

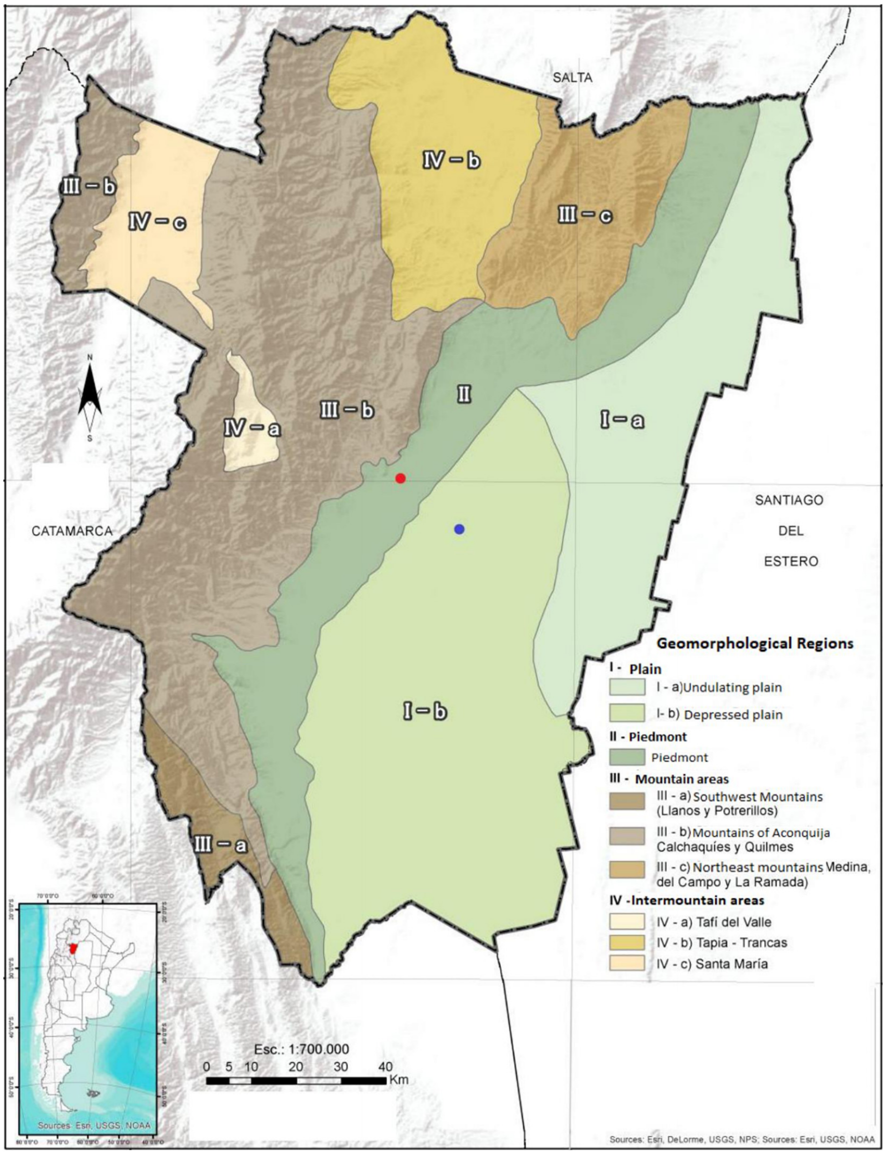

FIGURE 1 | Geographic location of Leales (in blue) and Famaillá (in red) apiaries. Map of Tucumán province with geomorphic divisions modified from Collantes and Busnelli (2014) [see lower left box for location of Tucuman province (red dot) on the map of Argentina].

(G5, G6, G8, G9, G11, G13)]; cubital index measured according to Ruttner (1988) (**Figure 2D**); and right hind wing (three internal lengths: wing L4, L5, and L9) (**Figure 2C**). Measurement of each body part were made from photographed images of glass slide preparations using ImageJ software (Schneider et al., 2012).

Geometric morphometry measurements were performed based on the right fore wing, which included 19 homologous, manually plotted, wing vein landmarks (**Figure 2E**). The digitized data of right fore wing preparations including the 19 wing landmarks were analyzed using the TPS package (version 1.46, Rohlf, 2010). Thirty-three variables were considered based on their utility and relevance in previously published studies consisting of 32 partial warp (pw) and the centroid size (Francoy et al., 2008). The nomenclature of each measurement was as follows: the term "pw" followed by the letter identifying the axis (*x*, *y*) and a number in series (pwx1, pwy1, pwx2, pwy2 . . . pwx16, pwy16).

Defensive Behavior

Parameters of defensive behavior were measured according to the scoring system developed by Ávalos et al. (2014). A score range from 1 to 4 (1 = the lowest intensity of response; 4 = the highest intensity of response) was assigned to the following behavioral parameters: "run" (tendency of worker bees to run on combs), "fly" (tendency of worker bees to fly off the combs during colony manipulations), "sting" (tendency of worker bees to hit the operator's veil), and "hang" (tendency of worker bees to be grouped). To measure the four parameters listed above, hives

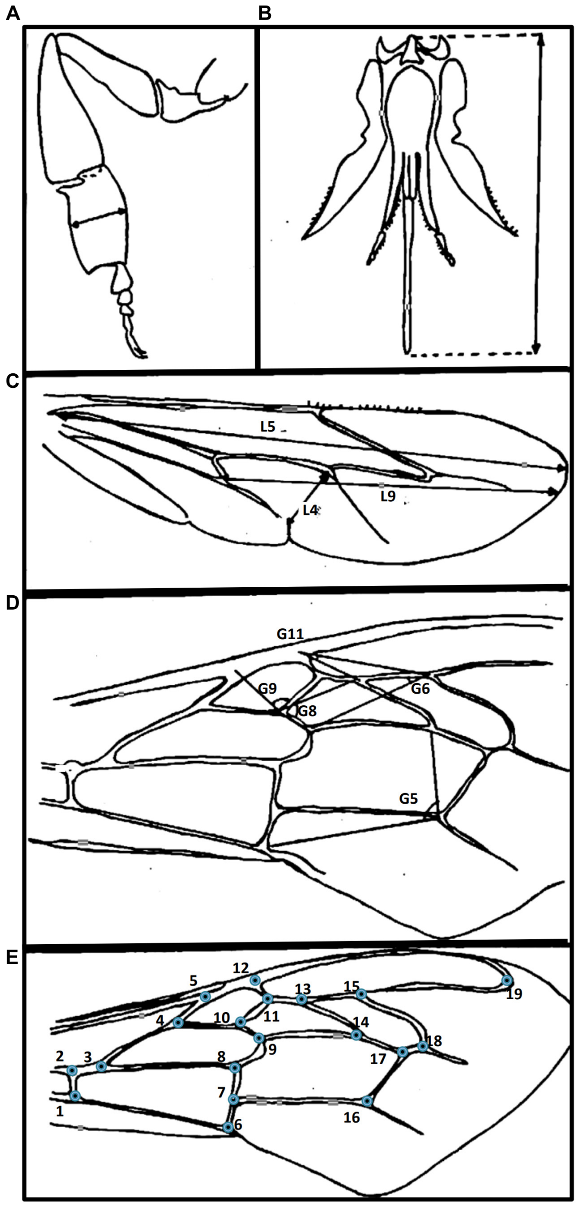

FIGURE 2 | Scheme of honey bee body parts used for morphometric analyses. In brackets variables measured in the present work. **(A–D)** Traditional morphometry: **(A)** right hind leg (metatarsal width); **(B)** mouthparts – proboscis (proboscis length); **(C)** right hind wing (three internal length); **(D)** right fore wing (five angles); and **(E)** geometric morphometry: right fore wing (19 landmarks plotted in the vein junctions).

were opened each time in the presence of the same observer (by applying a minimum amount of smoke per sting) followed by the direct observation of bee behaviors for a period of 30 s.

Statistical Analysis

The values of colony strength were compared between apiaries (LE and FAM) and time (mid-season, wintertime, and early spring) using the Kruskal–Wallis (K–W) test. *Post hoc* Dunn's test was applied for multiple comparisons. The data of brood population and phoretic *Varroa* were analyzed by two-way analysis of variance (ANOVA) (fixed factors = Apiary [LE and FAM] and Time [mid-season, wintertime, and early spring]; Apiary × Time). In the case of significant values for the interaction between factors, one-way ANOVAs and *post hoc* Tukey's HSD tests were performed. The lost colonies were excluded from the analyses.

As a first exploratory analysis of morphological variation between apiaries, bilateral Student's *T*-tests were separately carried out for all measured morphometric variables. Bonferroni adjustment for multiple comparisons was applied. Subsequently, all morphometric variables were analyzed by two-way ANOVA [fixed factors = Apiary (LE and FAM) and Haplotypes (A1 and A4); Apiary × Haplotype interaction]. C1 haplotype was dropped from the analysis because it was present in only one (LE) of the two evaluated apiaries. *Post hoc* Bonferroni test was applied for multiple comparisons. Before analyses, the variables were checked for normality with the Shapiro–Wilks test and for homogeneity of variances by Levene's test.

Defensive behavior variables were analyzed by apiary (LE and FAM) and time (mid-season, wintertime, and early spring). The lost colonies were excluded from the analysis. In addition, the same variables were analyzed by haplotype (A1 and A4) at mid-season. C1 haplotype was dropped from the analysis because it was present in only one (LE) of the two evaluated apiaries. All analyses were performed using K–W tests. *Post hoc* Dunn's test was applied for multiple comparisons.

A two-steps analysis was carried out to detect the most informative morphological and defensive behavior variables. First, a principal component analysis (PCA) was performed using all significant variables from the above mentioned statistical tests at mid-season, since all colonies exhibit the highest bee population at this time of the beekeeping season. For morphometric variables we included 10 samples (individuals) per colony. In the case of variables with only one value registered for the colony (defensive behavior) we considered the same value for all individuals from the same colony. A second multivariate analysis, a principal coordinate analysis (PCoA), was run using the Gower coefficient of similarity (Gower, 1971), considering selected variables from the PCA. The selection of morphometry and defensive behavior variables were performed according to their eigenvector coefficients for the two first components of the PCA (variables with a coefficient > 0.25 were selected). All analyses were performed using InfoStat 2016 statistical software (Di Rienzo et al., 2016) as well as SPSS 28.0 version (IBM Corporation 2010).

RESULTS

Honey bee colonies from LE and FAM apiaries (11 colonies in total: four from LE and seven from Famaillá; **Supplementary Table 1**) were evaluated from middle-productive season (spring-summer 2017) to early spring 2018. All colonies in both apiaries showed the highest strength at mid-season (category 1, criterion established for this survey). During wintertime, LE showed 80%

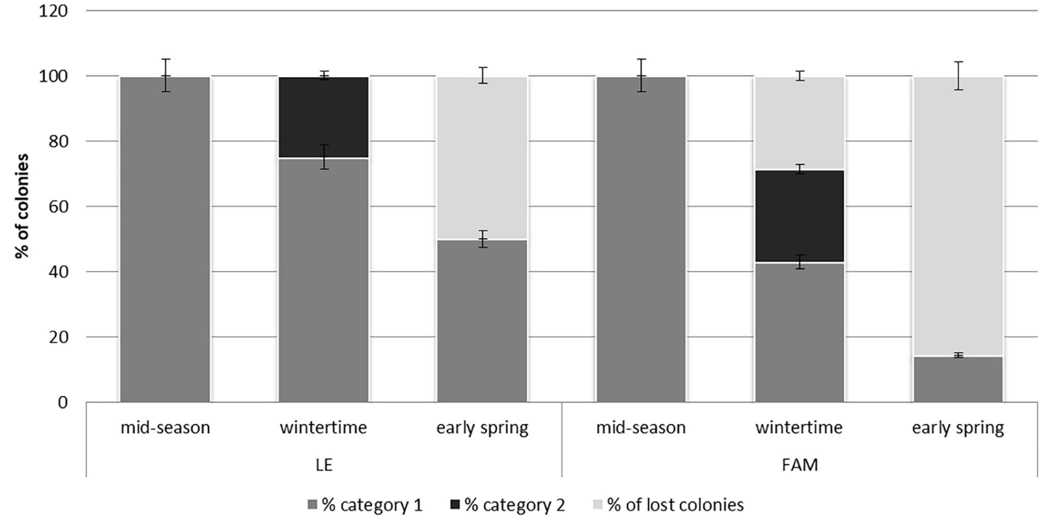

FIGURE 3 | Colony strength of *A. mellifera* colonies from Leales (LE) and Famaillá (FAM) apiaries during the beekeeping season (mid-season, wintertime and early spring). Mean percentage (±SE) of colonies in category 1 or 2 and percentage of lost colonies are shown.

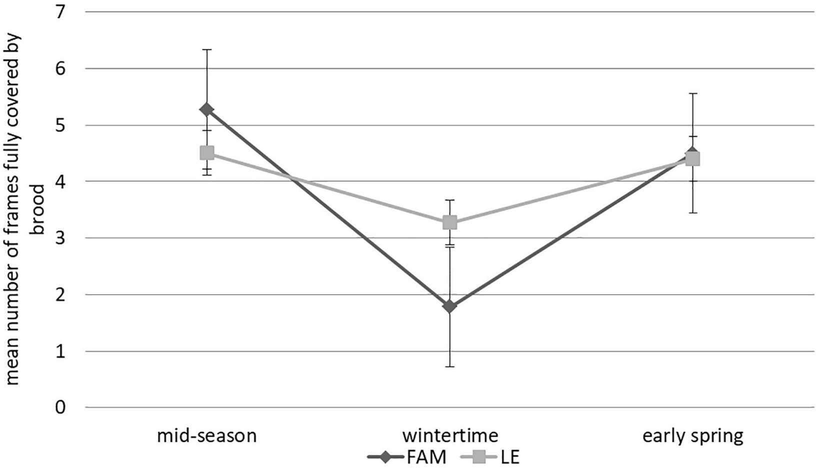

FIGURE 4 | Brood population of *A. mellifera* colonies from Leales (LE) and Famaillá (FAM) apiaries through the beekeeping season 2017–2018 (mid-season, wintertime and early spring). Mean values of number of frames fully covered by brood (±SE) are shown.

of the colonies (three colonies) in category 1 and 20% (one colony) in category 2, while FAM showed 42.8% of the colonies (three colonies) in category 1, 28.6% (two colonies) in category 2, and 28.6% of the colonies (two colonies) were lost. In early spring, 50% of the colonies (two colonies) in LE apiary were in category 1 and 50% (two colonies) were identified as lost colonies, while for FAM 14.3% of colonies (one colony) were in category 1 and the rest of the colonies were determined as lost (**Figure 3** and **Supplementary Table 1**). Non-significant differences were observed between apiaries ($H = 0.05$, $P = 0.71$ K–W test) neither among times of the season ($H = 1.75$, $P = 0.08$ K–W test) for colony strength.

Brood population showed similar patterns throughout the season, with no significant difference in the number of frames fully covered by brood between apiaries ($F_{(1,17)} = 0.0001$; $P = 0.99$; two-way ANOVA). However, significant differences were observed between different times of the season ($F_{(2,17)} = 26.24$; $p < 0.001$). Specifically, a significantly higher mean number of frames covered with brood was observed in mid-season (5.14 ± 0.27) compared to early spring (4.45 ± 0.52) and wintertime (2.33 ± 0.29) (*post hoc* comparison Tukey's HSD test) (**Figure 4** and **Supplementary Table 1**). A non-significant interaction between factors (Apiary and Time of the season) was observed ($F_{(2,17)} = 0.36$; $P = 0.70$).

The percentage of phoretic *Varroa* appeared to vary throughout the season in both apiaries, but with no significant differences ($F_{(2,17)} = 1.01$; $P = 0.38$; two-way ANOVA; **Figure 5**). The mean percentage of phoretic *Varroa* tended to be higher in mid-season (mean value for both apiaries: 7.05 ± 1.29%) than in early spring (mean value for both apiaries: 3.95 ± 2.52%),

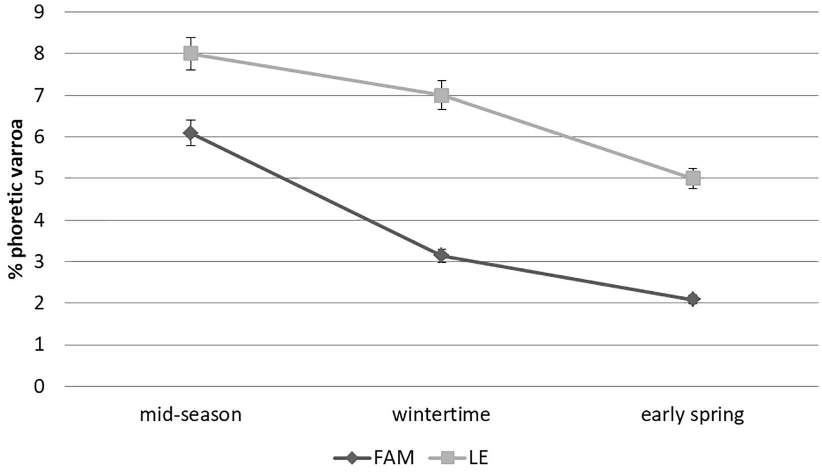

FIGURE 5 | Mean percentage of phoretic *Varroa* (±SE) of *A. mellifera* colonies from Leales (LE) and Famaillá (FAM) apiaries through the beekeeping season 2017–2018 (mid-season, wintertime and early spring).

with intermediate values during wintertime (mean value for both apiaries: 4.78% ± 1.38%) (**Figure 5**). Although the values observed in LE seemed to be higher than those in FAM throughout the season, non-significant differences between apiaries were detected ($F_{(1,17)} = 1.18$; $P = 0.20$). A non-significant interaction was observed between factors (Apiary and Time of the season) ($F_{(2,17)} = 0.24$; $P = 0.78$).

The genetic determination (COI-COII mitochondrial haplotypes) performed for all colonies from both apiaries (LE and FAM) showed that they were composed of 92% "A" and 8% "C" haplotypes. Within the "A" lineage, 50% corresponded to the A4 haplotype and the other 50% to the A1 haplotype. C1 was the only mitochondrial haplotype present within the "C" lineage (**Supplementary Table 1**).

A first exploratory analysis showed that 4 of 33 geometric morphometry variables and one of 12 traditional morphometry variables were significantly different between apiaries (Student's T-test, $P > 0.05$; **Supplementary Table 2**). Results obtained by the two-way ANOVA (Apiary; Haplotype; Apiary × Haplotype) showed that nine geometric morphometry variables and two traditional morphometry variables differed between apiaries (**Table 1A**). Specifically, higher values were observed for ProbL and G5 in LE compared with FAM (Bonferroni test; $P < 0.05$). The remaining traditional and geometric morphometry variables were not significantly different between apiaries.

Morphometric variables also differed between haplotypes. Ten of 33 geometric morphometry variables and two of 12 traditional morphometry variables showed significant differences between haplotypes (A1 and A4) (**Table 1B**). The remaining morphometric variables showed no statistically significant results ($P > 0.05$). For most of the analyzed variables, the two-way ANOVA showed that the Apiary × Haplotype interaction was not significant, thus differences in morphometric variables between apiaries were not influenced by haplotype. However, interactions (Apiary × Haplotype) resulted significant for L5, pwx8, and pwx12 morphometry variables. In these cases, differences in morphometric variables between apiaries depended on the haplotype detected. Specifically, the interaction analysis for L5 variable showed significant differentiation between the two haplotypes from LE apiary [A4 haplotype (LEA4) and A1 haplotype (LEA1)] while LEA4 and FAMA4 showed no significant difference. Pwx8 and pwx12 variables showed significant differentiation between A4 haplotype from LE (LEA4) vs. FAM (FAMA4), and also between LEA4/LEA1 and LEA4/FAMA1 in the case of pwx8 variable (see more details of comparisons in **Supplementary Table 3**).

Defensive behavior results showed that both apiaries displayed similar patterns for the four measured variables throughout the season (**Figure 6**). However, the K–W test showed significant differences between apiaries for "fly" ($H = 4.89$; $P = 0.027$). When times of the season were compared, significant differences were observed for "fly" and "sting," specifically, between wintertime and early spring ("fly" [$H = 11.83$; $P = 0.016$] and "sting" [($H = 6.73$; $P = 0.021$)]. In addition, border significant differences were observed for "hang" [$H = 10.00$; $P = 0.053$]; K–W test) for the same time comparison. LE showed higher values for "fly" and "hang" and lower values for "sting" compared to FAM (**Figure 6**). Defensive behavior showed non-significant differences for the four variables evaluated between haplotypes (A1 and A4) ($P > 0.05$; K–W test).

The PCA for combined morphometric and behavior variables showed that the first three components explained 78.3% of the variability among colonies (PC1 43.8%, PC2 21.5%, and PC3 13%; **Supplementary Table 4**). The distribution of honey bee colonies in the PCA space was mainly explained by the contribution of ProbL, pwy5, pwy6, and "fly," and associated with higher values on the PC1, while pwx4, pwx8, and pwy11 were main contributors to lower values on the PC1. In relation to PC2, the main positive contribution was due to the centroid size, pwx12 and G5, while lower values were associated with pwx3, pwy11, and pwx12 (**Figure 7** and **Supplementary Table 4**). The distribution of the colonies from each apiary in the plane (X-Y axes) was independent of mitochondrial haplotype (**Figure 7** and **Supplementary Table 4**). Specifically, LE3 (C1 haplotype)

TABLE 1 | (A) Geometric morphometry analysis.

Variable*	F	P
Centroid size	7.69	0.0066
pwx3	4.07	0.0465
pwy4	5.15	0.0255
pwy5	8.08	0.0055
pwy6	8.47	0.0045
pwx7	11.48	0.0010
pwx8	18.45	<0.0001
pwy11	9.56	0.0026
pwx12	7.23	0.0085
ProbL	29.35	<0.0001
G5	6.63	0.0115

(B) Geometric morphometry analysis.

Variable*	F	P
Centroid size	5.96	0.0036
pwx2	6.75	0.0108
pwx3	6.83	0.0104
pwx4	6.87	0.0102
pwx7	4.07	0.0465
pwx8	7.28	0.0082
pwx11	5.16	0.0254
pwy11	5.89	0.0171
pwy13	4.20	0.0432
pwx14	8.35	0.0048
L5	5.42	0.0220
G5	6.88	0.0101

*(A) Comparisons between LE and FAM apiaries. *Only variables with significant results between apiaries were shown (two-way ANOVA; F: Fisher statistic (F[1,96]); P: P-value of significance.*

*(B) Comparisons among mitochondrial haplotypes (A1 and A4). *Only variables with significant results were showed (two-way ANOVA; F: Fisher statistic (F[2,96]); P: P-value of significance.*

was positioned in the center of the graph, while (FAM 3, 4, 6, 8 and LE 4 with A4 haplotype) and (FAM 9, 5, 7 and LE 5, 2) were distributed throughout the two-dimensional space without a clear association between variables and haplotypes. Moreover, the PCoA using all 12 characters previously selected from the PCA (variables with coefficient values > 0.25; **Supplementary Table 4**) explained 48.1% of the variability among colonies in the first two coordinates. Colony characteristics are mostly related to the location (apiary) rather than the haplotype, showing the grouping of those belonging to Famaillá on the left and those belonging to Leales on the right, with the exception of the FAM6 and LE3 which are in the middle (**Supplementary Figure 1**). The pattern observed in the scatterplots (PCA and PCoA) showed that colonies belonging to FAM were partially separated from those of LE.

DISCUSSION

The information obtained in the present study allowed us to explore the relevance of morphometric and behavioral variables as indirect indicators of the potential adaptation of AHB populations to subtropical agro-ecological regions of Argentina. Moreover, this study brings valuable information for the characterization and preservation of Africanized populations and supports the need for honey bee breeding programs established at a regional level.

Our study reports similar dynamics between apiaries for colony characteristics, mainly adjusted to the high availability of food and similar environmental nectar influx levels. Brood population remains more stable for LE than FAM throughout the beekeeping season, with the latter showing an abrupt drop at the end of the winter. These results in the wintertime could be due to different nutritional status between apiaries for the two agro-ecological regions and associated with food availability or quality. Previous research has proposed that availability of pollen, nectar reserves and quality of stored pollen are the principal reasons for decreases in brood population. Moreover, other studies have shown that *A. mellifera* can modulate its reproductive rate according to limiting environmental resources, such as availability of food (Le Conte and Navajas, 2008). An example of this adaptive process is the decline of brood as food reserves are depleted. In accordance with this, our results showed colony losses during the winter and early spring, but the absence of colonies presenting an intermediate population size, in the previous inspection, during the mid-season. This could be explained by the strong tendency of AHBs to swarm (Guzmán-Novoa et al., 2011; Uzunov et al., 2014) in the presence of stress factors such as lack of food availability, invasive insects in the hive (such as, ants, and beetles), among other environmental variables.

The colony evaluations performed in the present study showed the absence of significant differences between apiaries with respect to *Varroa* levels, which were found to be higher than the expected phoretic *Varroa* values of the region (5% for mite-treated colonies; UDA Los Sarmientos, 2021). This can be attributed to almost all the remaining colonies being naturally tolerant to the mite (with a relatively high load of phoretic *Varroa* during mid-season), as expected from their Africanized origin. The natural tolerance of AHBs to high *Varroa* infestation has been previously described (Schneider et al., 2004; Guzmán-Novoa et al., 2011). In line with our results on apiaries located in subtropical climates, Medina-Flores et al. (2014) reported no differences in the levels of infestation between Africanized and pure European colonies from Mexico.

The analysis of morphometric variables showed significant differences between Famaillá and Leales apiaries. However, only a few traditional morphometry characters were variable. A recent study also demonstrated low levels of differentiation among bee ecotypes using traditional wing length morphometry (Calfee et al., 2020). Present results showed that proboscis length could be used to differentiate apiaries. This variation could be linked to the floral species present in the adjoining areas and to the availability of food, and a reflection of the influence of the differential agro-ecological zone where the populations were located. The environmental characteristics of both regions have been previously described. The Depressed Plain region, location of Leales apiary, is an agricultural region represented by the

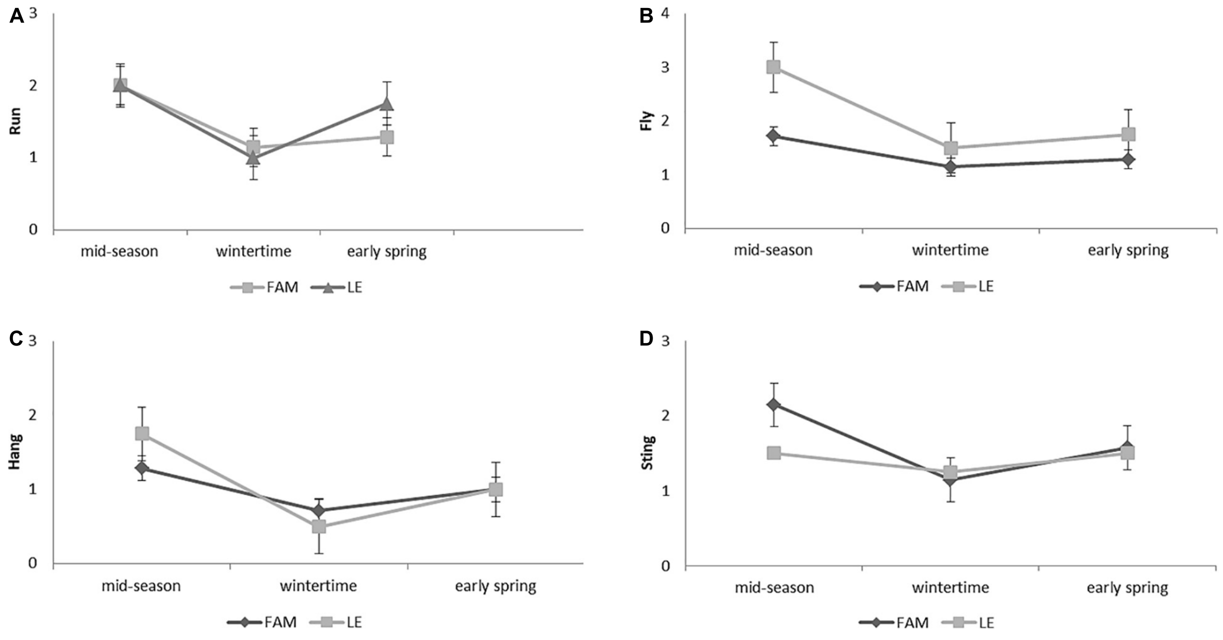

FIGURE 6 | Defensive behavior of *A. mellifera* colonies from Leales (LE) and Famaillá (FAM) apiaries through the beekeeping season (2017–2018). Mean values (±SE) for "run" **(A)**; "fly" **(B)**; "hang" **(C)**; and "sting" **(D)** are shown.

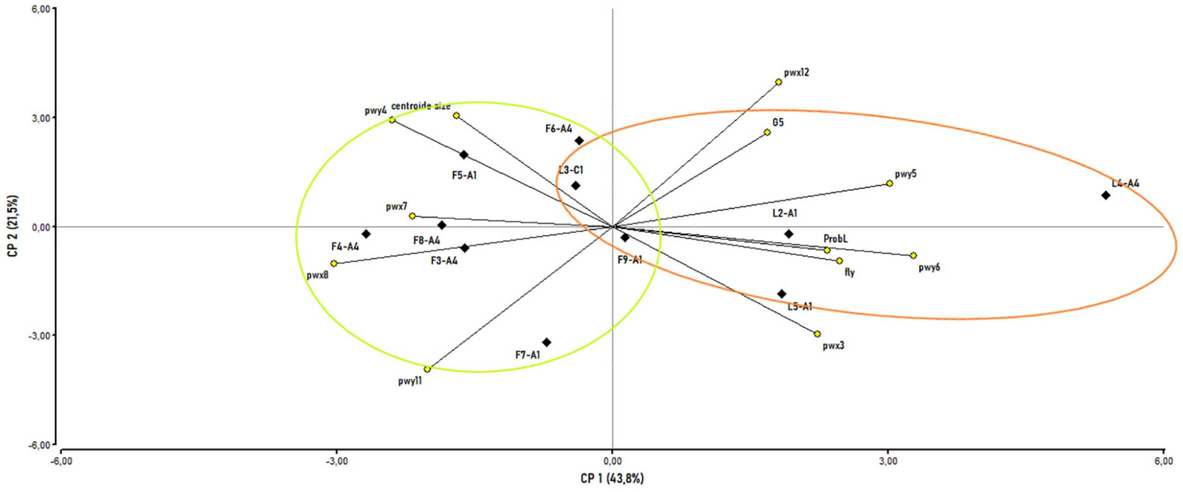

FIGURE 7 | Principal components analysis (PCA) scatterplot for colonies based on morphometric and behavioral variables. Morphological measurements included: ProbL, G5, centroid size, pwx3, pwy4, pwy5, pwy6, pwx7, pwx8, pwy11, and pwx12. Behavioral measurements included: "fly." Mitochondrial haplotypes are: A1, A4, and C1. The colonies were named according to the apiary they belong (L: LE and F: FAM) followed by the number of colony – Haplotype, according to the information described in **Supplementary Table 1**. Ellipses show colonies from the same apiary. Green ellipse: FAM; yellow ellipse: LE.

cultivation of sugar cane, soybean and corn with the presence of native plant species of the Chaco Serrano type (Cruzate et al., 2005; Benedetti et al., 2019), while in the Piedmont region, location of Famaillá apiary, major agricultural activity is based on the cultivation of citrus fruits and sugar cane (Benedetti et al., 2019). The latter region is also characterized by the presence of native plant species belonging to the group of Yungas (Cruzate et al., 2005). Currently there is no published information on the characteristics and use of floral resources by honey bees in these regions, hence a detailed characterization of floral shape, structure and composition of each agro-ecological regions of Argentina would be necessary to test the hypothesis of the rapid adaptation of honey bees to their environment. In this regard, previous studies have predicted a rapid dispersion and adaptation of AHBs to new habitats (Cox, 1994; Rivera-Marchard et al., 2012; Ackerman, 2021). A rapid evolution can generate major changes to characteristics such as morphological or behavioral traits, as occurred with the defensive behavior in the gentle

AHBs from Puerto Rico (Avalos et al., 2017). Similar results have been described for honey bee ecotypes from Eastern Europe, for which the proboscis length varies across a climatic cline and as a consequence of a broad hybrid zone of *A. mellifera* subspecies from this region (Alpatov, 1929; Ruttner, 1952; Meixner et al., 2007). Further phenotypic and genomic analysis is necessary to determine if a rapid adaptation of AHB has occurred in South America, specifically in different agro-ecological regions.

Based on the results from our morphometric analyses, geometric morphometry showed greater sensitivity than traditional morphometry to detect significant differences between apiaries. A positive relationship between geometric morphometry variables and apiary location could indicate the presence of an environmental effect, as well as other factors not directly assessed in this study (such as parental gene effects). In addition, we found that sensitivity decreases when analyzing morphometric differences between haplotypes, as non-significant differences were detected, in accordance with recent results by Porrini et al. (2020). These results could be due to phenotypic similarity between A4 and A1 and the ongoing hybridization process present in the northern region of Argentina, and suggest that geometric morphometry would not be sufficiently sensitive as an indirect marker of mitochondrial haplotype for these populations, as proposed by Kandemir et al. (2011) for other populations.

We detected differences in defensive behavior, specifically for "fly" behavior, between apiaries independent of mitochondrial haplotype. We conclude this to likely be the result of paternal and/or environmental effects. It is worth noting that the apiaries being compared in this study had different drone congregation areas. As it has previously been described, this can affect the genetic composition/variability of surrounding apiaries (Collet et al., 2009; Galindo-Cardona et al., 2017, 2020). The paternal effect on defensive behavior has also been previously described (De Grandi-Hoffman et al., 1998; Guzman-Novoa et al., 2005). The study of drone contribution to the genetic makeup of apiaries in both agro-ecological regions of Tucuman province is important to document local variations in honey bee defensive behavior as this can be utilized to identify methods and tools for the selection of bee stocks adapted to specific regional environments for breeding programs.

Environmental effects on honey bee defensive behavior have been addressed by several authors (Buco et al., 1986; Southwick and Moritz, 1987; Rivera-Marchard et al., 2012; Nouvian et al., 2016). In particular, Rivera-Marchard et al. (2012) described the importance of climate to the level of defensive behavior response, including the effect of food reserves, and found that the more limited the food, the greater the response. Along similar lines of thought, Scofield and Mattila (2015) proposed that quality and abundance of pollen that honey bee larvae feed on can affect adult behavior. Other authors have described how lack of nutrients and body reserves can affect the health of the colony, which can become vulnerable to *Nosema ceranae* and *V. destructor*, increasing colony stress (Invernizzi et al., 2011). Moreover, the impact of agricultural activities in the vicinity of apiaries has been explored, including agro-chemicals as a major factor negatively affecting honey bee populations (Brown and Paxton, 2009). In our study, we observed lower values of the defensive behavior variables during wintering compared to the productive season. This result is likely associated with the combined effect of limited food resources and decreased number of individuals inside the colony during wintertime. Further analyses, considering agricultural activities in surrounding areas and the detection of potential honey bee stressors in the colony assessment will be useful to evaluate the environmental impact on the defensiveness of locally-adapted honey bees in agricultural settings.

The analysis using the COI-COII mitochondrial region was consistent with recently published works (Agra et al., 2018; Calfee et al., 2020; Porrini et al., 2020) that described the presence of genetic variability and a preponderant presence of African lineages (A1 and A4) in feral honey bee colonies mainly established in the north of Argentina. However, a saturation of AHBs in the north of Argentina previously described by Sheppard et al. (1991) was not observed in our study. In addition, Agra et al. (2018) found a predominance of European mitochondrial haplotypes in commercial apiaries, and of A4 and A1 haplotypes in colonies of wild origin from the north of Argentina. Our results on the genetic assessment of wild-origin honey bee colonies are congruent with the mentioned previous studies, as A1 and A4 haplotypes were also detected in a high frequency.

Results from this study revealed significant differences in morphometric and defensive behavior variables between haplotypes, supporting their potential utility for the selection of honey bee stocks with low defensiveness. Furthermore, our results showed that the feral colonies tested have retained some morphometric and defensive behavior characteristics of their African origin, according to the traits previously described by several authors (Breed et al., 2004; Schneider et al., 2004; Francoy et al., 2008; Guzmán-Novoa et al., 2011; Rivera-Marchard et al., 2012).

The combined evaluation of morphometric and defensive behavior variables, performed in our study by multivariate analyses (PCoA and PCA), indicates that maternal lineage (mt haplotype) is not a determining factor explaining the variation among colonies. However, the variation is best explained by colony location (apiary). This could be partially explained by the high hybridization rate of AHB populations in Northern Argentina and the strong influence of the paternal lineage on defensive behavior as previously described by Clarke et al. (2002) and Guzman-Novoa et al. (2005). The multivariate analyses also indicate that some morphometric measures and defensive behavior traits used in this study could be of future utility to track differences in colony response to different agro-ecological regions.

CONCLUSION

This study determined differences in morphological and behavioral characteristics of honey bees from apiaries located in different agro-ecological zones of Northwest Argentina. The environment, type of farming and agricultural products of each area could potentially impact the nutritional status of *A. mellifera* colonies and affect access to nutrients from crops and native vegetation. In line with this, we conclude that the morphological and behavioral differences observed between apiaries could be associated with the adaptation of honey bees to specific resources available in the different agro-ecological regions examined.

The study also highlighted the possible role played by feral drone matings in determining the diversity of honey bee population in the regions examined. Further studies of the paternal contribution of the nearest drone congregation areas, using nuclear molecular genetic markers, will bring valuable higher resolution information and guidance to breeding programs of locally adapted honey bee ecotypes.

As a means to support local sustainable apiculture and the preservation of AHBs, this work presents initial findings from a comparison between honey bee populations from different agro-ecological zones of Northwest Argentina and tools that can be used to characterize honey bee ecotypes with desirable characteristics, such as, low defensiveness and tolerance to *Varroa.*

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

EB, GR, MP, and SL conceived and designed the experiments. EB performed the statistical analyzes. MA participated in the genetic analysis. AS participated in the statistical analyses. EB, GG, and LM participated in the survey and contributed to live material. CG participated in the morphometric measurements. EB, AS, and SL wrote the manuscript. All the authors read and accepted the manuscript, contributed to the article, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.590225/full#supplementary-material

Supplementary Figure 1 | Principal coordinate analysis (PCoA) scatterplot for colonies based on selected morphometric and behavioral variables (Gower distances). Twelve variables were included in this analysis (selected according to their coefficient values in PCA; **Supplementary Table 4**) as follows: ProbL, G5, centroid size, pwy3, pwy4, pwy5, pwy6, pwx7, pwx8, pwy11, pwx12, and "fly." The colonies were named according to the apiary they belong (L: LE and *F*: FAM) followed by the number of colony – Haplotype, according to the information described in **Supplementary Table 1**.

Supplementary Table 1 | Characteristics of Leales (LE) and Famaillá (FAM) colonies in the mid-season.

Supplementary Table 2 | Student's *T*-test for morphometric variables between apiaries (FAM and LE). *Only variables with significant results ($P < 0.05$) between apiaries were shown. *T*: *T*-test statistic; $n_{FAM} = 70$; $n_{LE} = 40$. *P*: *P*-value of significance. *P**: adjusted *P*-value (Bonferroni corrections for multiple comparisons).

Supplementary Table 3 | Two-way ANOVA – Interaction analysis (Apiary × Haplotype) for morphometry variables. Only statistically significant results ($P < 0.05$) were shown.

Supplementary Table 4 | Principal component analysis (PCA) of colonies for morphometric and defensive behavior variables. Eigenvalues and eigenvectors.

REFERENCES

Acevedo-Gonzalez, J. P., Galindo-Cardona, A., Avalos, A., Whitfield, C. W., Rodriguez, D. M., Uribe-Rubio, J. L., et al. (2019). Colonization history and population differentiation of the Honey Bees (*Apis mellifera* L.) in Puerto Rico. *Ecol. Evol.* 9, 10895–10902.

Ackerman, J. D. (2021). Island Invasions by Introduced Honey Bees: what Can Be Expected for Puerto Rico and the Caribbean? *Front. Ecol. Evol.* 8:556744. doi: 10.3389/fevo.2020.556744

Agra, M. N., Conte, C. A., Corva, P. M., Cladera, J. L., Lanzavecchia, S. B., and Palacio, M. A. (2018). Molecular characterization of *Apis mellifera* colonies from Argentina: genotypic admixture associated with ecoclimatic regions and apicultural activities. *Entomol. Exp. Appl.* 166, 724–738. doi: 10.1111/eea.12719

Alaux, C., Sinha, S., Hasadsri, L., Hunt, G. J., Guzman-Novoa, E., DeGrandi-Hoffman, G., et al. (2009). Honey bee aggression supports a link between gene regulation and behavioral evolution. *Proc. Natl. Acad. Sci.* 106, 15400–15405. doi: 10.1073/pnas.0907043106

Alpatov, W. W. (1929). Biometrical studies on variation and races of the honeybee *Apis mellifera* L. *Q. Rev. Biol.* 4, 1–57.

Andere, C., García, C., Marinelli, C., Cepeda, R., Rodríguez, E. R., and Palacio, M. A. (2008). Morphometric variables of honeybees *Apis mellifera* used in ecotypes characterization in Argentina. *Ecol. Model.* 214, 53–58. doi: 10.1016/j.ecolmodel.2008.01.010

Avalos, A., Pan, H., Li, C., Acevedo-Gonzalez, J. P., Rendon, G., Fields, C. J., et al. (2017). A soft selective sweep during rapid evolution of gentle behaviour in an Africanized honeybee. *Nat. Commun.* 8:1550.

Ávalos, A., Rodríguez-Cruz, Y., and Giray, T. (2014). Individual responsiveness to shock and colony-level aggression in honey bees: evidence for a genetic component. *Behav. Ecol. Sociobiol.* 68, 761–771. doi: 10.1007/s00265-014-1689-8

Bedescarrabure, E. (2011). *Consolidando la Apicultura como herramienta de desarrollo*. United States: *INTA*.

Benedetti, P. E., Sopena, R. A., Morales, C. C., and Moreno, R. A. (2019). *Caña de azúcar: tucumán redujo un 5,4% la superficie plantada con respecto a 2018*. United States: *INTA*.

Bierzychudek, A. (1979). *Historia De La Apicultura Argentina*. Argentina: Universidad Nacional de Córdoba. doi: 10.1017/CBO9781107415324.004

Breed, M. D., Guzmán-Novoa, E., and Hunt, G. J. (2004). Defensive behavior of honey bees: organization, Genetics, and Comparisons with Other Bees. *Annu. Rev. Entomol.* 49, 271–298. doi: 10.1146/annurev.ento.49.061802.123155

Brown, M. J. F., and Paxton, R. J. (2009). The conservation of bees: a global perspective. *Apidologie* 40, 410–416. doi: 10.1051/apido/2009019

Buco, S. M., Rinderer, T. E., Sylvester, H. A., Collins, A. M., Lancaster, V. A., and Crewe, R. M. (1986). Morphometric differences between South American Africanized and South African (*Apis mellifera scutellata*) honey bees. *Apidology* 18, 217–222.

Calfee, E., Agra, M. N., Palacio, M. A., Ramírez, S. R., and Coop, G. (2020). Selection and hybridization shape the Africanized honey bee invasion of the Americas. *BioRxiv* 17:994632. doi: 10.1101/2020.03.17.994632

Clarke, K. E., Rinderer, T. E., Franck, P., Quezada-Euán, J. G., and Oldroyd, B. P. (2002). The Africanization of honeybees (Apis mellifera L.) of the Yucatan: a study of a massive hybridization event across time. *Evolution* 56, 1462–1474. doi: 10.1111/j.0014-3820.2002.tb01458.x

Collantes, M., and Busnelli, J. (2014). *Geología de Tucumán, Colegio de Graduados en Ciencias Geológicas de la Provincia de Tucuman*. Argentina: INSUGEO. 157–169.

Collet, T., Ferreira, K. M., Arias, M. C., Soares, A. E. E., and Del Lama, M. A. (2006). Genetic structure of Africanized honeybee populations (*Apis mellifera* L .) from Brazil and Uruguay viewed through mitochondrial DNA COI – COII patterns. *Heredity* 97, 329–335. doi: 10.1038/sj.hdy.6800875

Collet, T., Santos Cristino, A., Prada Quiroga, C. F., Soares, A. E. E., and Del Lama, M. A. (2009). Genetic structure of drone congregation areas of Africanized honeybees in southern Brazil. *Genet. Mol. Biol.* 32, 857–863. doi: 10.1590/S1415-47572009005000083

Cox, B. (1994). AHB in Puerto Rico. *Am. Bee J.* 134:668.

Cruzate, G. A., Moscatelli, G. N., and Panigatti, L. (2005). *Suelos y Ambientes Tucumán-Argentina*. Available online at: http://www.geointa.inta.gob.ar/wp-ontent/uploads/downloads/Laminas_de_Suelos/Tucuman_3.jpg (accessed May 20, 2020).

De Grandi-Hoffman, G., Collins, A., Martin, J. H., Schmidt, J. O., and Spangler, H. G. (1998). Nest defense behavior in colonies from crosses between Africanized and European honey bees (*Apis mellifera* L.) (Hymenoptera: Apidae). *J. Insect Behav.* 11, 37–45. doi: 10.1023/A:1020862432087

De Jong, R., Morse, R. A., and Eickwort, G. C. (1982). Mites pests of honey bees. *Annu. Rev. Entomol.* 27, 229–252.

Di Rienzo, J. A., Casanoves, F., Balzarini, M. G., Gonzales, L., Tablada, M., and Robledo, C. W. (2016). *InfoStat versión 2018*. Argentina: Grupo InfoStat, FCA, Universidad Nacional de Córdoba.

Dietemann, V., Nazzi, F., Martin, S. J., Anderson, D., Locke, B., Keith, S. D., et al. (2013). The COLOSS BEEBOOK, Volume II: standard methods for *Apis mellifera* pest and pathogen research. *J. Apicul. Res.* 52. doi: 10.3896/IBRA.1.52.1.09

Dini, C., and Bedescarrabure, E. (2011). *Manual De Apicultura Para Ambientes Subtropicales*. United States: *Inta*

Figini, E. E., Frigoli, L. R., Poffer, D., and Barreto, J. A. (2017). *Apicultura: categorización de Colmenas*. United States: Inta. Available online at: https://inta.gob.ar/documentos/apicultura-categorizacion-de-colmenas (accessed June 10, 2020).

Franck, P., Garnery, L., Celebrano, G., Solignac, M., and Cornuet, J. M. (2000). Hybrid origins of honeybees from Italy (*Apis mellifera ligustica*) and Sicily (*A. m. sicula*). *Mol. Ecol.* 9, 907–921. doi: 10.1046/j.1365-294X.2000.00945.x

Francoy, T. M., Wittmann, D., Drauschke, M., Muller, S., Steinhage, V., Marcela, A. F., et al. (2008). Identification of Africanized honey bees through wing morphometrics: two fast and efficient procedures. *Apidologie* 39, 488–494. doi: 10.1051/apido:2008028

Galindo-Cardona, A., Ayup, M., Quiroga, O., and Bianchi, E. M. (2017). Primer reporte de un Área de Congregación de Zánganos de *Apis mellifera* (Hymenoptera: Apidae) de Argentina. *Rev. Soc. Entomol. Argent.* 76, 50–53. doi: 10.25085/rsea.761207

Galindo-Cardona, A., Scannapieco, A. C., Russo, R., Escalante, K., Geria, M., Lepori, N., et al. (2020). Varroa destructor Parasitism and Genetic Variability at Honey Bee (*Apis mellifera*) Drone Congregation Areas and Their Associations With Environmental Variables in Argentina. *Front. Ecol. Evol.* 8:590345. doi: 10.3389/fevo.2020.590345

Gower, J. (1971). A general coefficient of similarity and some of its properties. *Biometrics* 27, 857–871.

Guzmán-Novoa, E., Espinoza-Montaño, L. G., Correa-Benítez, A., and Guzmán-Novoa, G. (2011). Colonization, impact and control of Africanized honey bees in Mexico. *Vet. Mex.* 42, 149–178.

Guzman-Novoa, E., Hunt, G. J., Page, R. E., Uribe-Rubio, J. L., Prieto-Merlos, D., and Becerra-Guzman, F. (2005). Paternal effects on the defensive behavior of honeybees. *J. Hered.* 96, 376–380. doi: 10.1093/jhered/esi038

Guzmán-Novoa, E., and Page, R. E. (1999). Selective breeding of honey bees (Hymenoptera: Apidae) in Africanized areas. *J. Econ. Entomol.* 92, 521–525. doi: 10.1093/jee/92.3.521

Hall, H. G., and Smith, D. R. (1991). Distinguishing African and European honeybee matrilines using amplified mitochondrial DNA. *Proc. Natl. Acad. Sci. U. S. A.* 88, 4548–4552. doi: 10.1073/pnas.88.10.4548

Hunt, G. J. (2007). Flight and fight: a comparative view of the neurophysiology and genetics of honey bee defensive behavior. *J. Insect Physiol.* 53, 399–410. doi: 10.1016/j.jinsphys.2007.01.010

Invernizzi, C., Santos, E., García, E., Daners, G., Di Landro, R., Saadoun, A., et al. (2011). Sanitary and nutritional characterization of honeybee colonies in *Eucalyptus grandis* plantations. *Arch. Zoot.* 60, 1303–1314. doi: 10.4321/s0004-05922011000400045

Iqbal, J., Ali, H., Owayss, A. A., Raweh, H. S. A., Engel, M. S., Alqarni, A. S., et al. (2019). Olfactory associative behavioral differences in three honey bee *Apis mellifera* L. races under the arid zone ecosystem of central Saudi Arabia. *Saudi J. Biol. Sci.* 26, 563–568. doi: 10.1016/j.sjbs.2018.08.002

Kandemir, I., Özkan, A., and Fuchs, S. (2011). Reevaluation of honeybee (*Apis mellifera*) microtaxonomy: a geometric morphometric approach. *Apidologie* 42, 618–627. doi: 10.1007/s13592-011-0063-3

Kent, R. B. (1988). The Introduction and Diffusion of the African Honeybee in South America. *Assoc. Pacif. Coast Geogr.* 50, 21–43. doi: 10.4324/9780429490323-1

Kerr, W. E., and Nielsen, R. A. (1967). Sex determination in bees (apinae). *J. Apicult. Res.* 6, 3–9. doi: 10.1080/00218839.1967.11100154

Le Conte, Y., and Navajas, M. (2008). Climate change: impact on honey bee populations and diseases. *Rev. Sci. Tech.* 27, 485–497. doi: 10.20506/rst.27.2.1819

Lobo Segura, J. A. L. (2000). Highly polymorphic DNA markers in an Africanized honey bee population in Costa Rica. *Genet. Mol. Biol.* 23, 317–322. doi: 10.1590/S1415-47572000000200013

Los Sarmientos, U. D. A. (2021). Resultados Técnicos 20/21, Red de Unidades Demostrativas Apícolas – Programa de Apicultura PROAPI, RedLAC. Disponible en. Available online at: https://inta.gob.ar/documentos/unidades-demostrativas-apicolas (accessed July 23, 2021).

Medina-Flores, C. A., Guzmán-Novoa, E., Hamiduzzaman, M. M., Aréchiga-Flores, C. F., and López-Carlos, M. A. (2014). Africanized honey bees (*Apis mellifera*) have low infestation levels of the mite *Varroa destructor* in different ecological regions in Mexico. *Genetics and Molecular Research* 13, 7282–7293. doi: 10.4238/2014.February.21.10

Meixner, M. D., Pinto, M. A., Bouga, M., Kryger, P., Ivanova, E., and Fuchs, S. (2013). Standard methods for characterising subspecies and ecotypes of *Apis mellifera*. *J. Apic. Res.* 52. doi: 10.3896/IBRA.1.52.4.05

Meixner, M. D., Worobik, M., Wilde, J., Fuchs, S., and Koeniger, N. (2007). *Apis mellifera mellifera* in eastern Europe — morphometric variation and determination of its range limits. *Apidologie* 38, 191–197. doi: 10.1051/apido:2006068

Navajas, M., Migeon, A., Alaux, C., Martin-Magniette, M. L., Robinson, G. E., Evans, J., et al. (2008). Differential gene expression of the honey bee *Apis mellifera* associated with *Varroa destructor* infection. *BMC Genom.* 9:301. doi: 10.1186/1471-2164-9-301

Nouvian, M., Reinhard, J., and Giurfa, M. (2016). The defensive response of the honeybee *Apis mellifera*. *J. Exp. Biol.* 219, 3505–3517. doi: 10.1242/jeb.143016

Padilla, A. F., Reyes López, J., and Hernández Fernández, R. (2001). Estudio biométrico de la abeja melifera (*Apis mellifera*, Linneo 1758) (Hymenoptera, Apidae) de la isla de La Palma del Archipiélago Canario. II. Ángulos y longitudes de las alas. *Zool. Baet.* 12, 23–36.

Palacio, M. A., Figini, E., Rodriguez, G. A., Ruffinengo, S., and Bedescarrabure, E. (2000). Changes in a population of *Apis mellifera* selected for its hygienic behaviour. *Apidologie* 31, 471–478.

Porrini, L. P., Porrini, M. P., Garrido, M. P., Muller, F., Arrascaeta, L., Iriarte, P. F., et al. (2020). Infectivity and virulence of *Nosema ceranae* (Microsporidia) isolates obtained from various *Apis mellifera* morphotypes. *Entomol. Exp. Appl.* 68, 1–9. doi: 10.1111/eea.12902

Rivera-Marchand, B., Oskay, D., and Giray, T. (2012). Gentle Africanized bees on an oceanic island. *Evout. Appl.* 5, 746–756. doi: 10.1111/j.1752-4571.2012.00252.x

Rohlf, F. J. (2010). *Tpsrelw: relative Warps Analysis. Version 1.49*. New York: State University of New York at Stony Brook.

Ruttner, F. (1952). Alter und Herkunft der Bienenrassen Europas, *Österr. Imker* 2, 8–10.

Ruttner, F. (1988). *Biogeography and Taxonomy of Honeybees*. Berlin: Springer-Verlag. doi: 10.1007/978-3-642-72649-1

Salizzi, E. (2014). Reestructuración económica y transformaciones en el agro pampeano: la expansión del cultivo de la soja y sus efectos sobre la apicultura bonaerense en los inicios del siglo XXI. *Rev. Geogr.* 16, 13–46.

Schneider, C., Rasband, W., and Eliceiri, K. (2012). NIH Image to ImageJ: 25 years of image analysis No Title. *Nauret Methods* 9, 671–675. doi: 10.1038/nmeth.2089

Schneider, S., De Grandi-Hoffman, G., and Smith, D. R. (2004). The African honey bee: factors Contributing to a Successful Biological Invasion. *Annu. Rev. Entomol.* 49, 351–376. doi: 10.1146/annurev.ento.49.061802.123359

Scofield, H. N., and Mattila, H. R. (2015). Honey bee workers that are pollen stressed as larvae become poor foragers and waggle dancers as adults. *PLoS One* 10, 1–19. doi: 10.1371/journal.pone.0121731

Sheppard, W. S., Rinderer, T. E., Garnery, L., and Shimanuki, H. (1999). Analysis of Africanized honey bee mitochondrial DNA reveals further diversity of origin. *Genet. Mol. Biol.* 22, 73–75.

Sheppard, W. S., Rinderer, T. E., Mazzoli, J. A., Stelzer, J. A., and Shimanuki, H. (1991). Gene flow between African-and European-derived honey bee populations in Argentina. *Nature* 349, 782–784.

Southwick, E. E., and Moritz, R. F. A. (1987). Effects of Meteorological Factors on Defensive Behaviour of Honey Bees. *Int. J. Biometeorol.* 31, 259–265.

Unger, N., Poffer, D., Frigoli, L., Marcó, O., and Fourquet, G. (2013). *Manual De Prácticas Apícolas Para Producir Miel de Calidad en la Cuenca Del Salado*. United States: INTA.

Uzunov, A., Costa, C., Panasiuk, B., Meixner, M., Kryger, P., Fani, H., et al. (2014). Swarming, defensive and hygienic behaviour in honey bee colonies of different genetic origin in a pan-European experiment. *J. Apic. Res. Bee World* 53, 248–260.

Van Engelsdorp, D., and Meixner, M. D. (2010). A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. *J. Inverteb. Pathol.* 103, S80–S95. doi: 10.1016/j.jip.2009.06.011

Whitfield, C. W., Ben-Shahar, Y., Brillet, C., Leoncini, I., Crauser, D., Leconte, Y., et al. (2006). Genomic dissection of behavioral maturation in the honey bee. *Proc. Natl. Acad. Sci. U. S. A.* 103, 16068–16075. doi: 10.1073/pnas.0606909103

Zuccardi, R. B., and Fadda, G. S. (1985). *Bosquejo agrológico de la provincia de Tucumán*. Argentina: Universidad Nacional de Tucumán.

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REVIEW
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The Movement of Western Honey Bees (*Apis mellifera* L.) Among U.S. States and Territories: History, Benefits, Risks, and Mitigation Strategies

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Beekeeping is a cornerstone activity that has led to the human-mediated, global spread of western honey bees (*Apis mellifera* L.) outside their native range of Europe, western Asia, and Africa. The exportation/importation of honey bees (i.e., transfer of honey bees or germplasm *between* countries) is regulated at the national level in many countries. Honey bees were first imported into the United States in the early 1600's. Today, honey bee movement (i.e., transport of honey bees *among* states and territories) is regulated within the United States at the state, territory, and federal levels. At the federal level, honey bees present in the country (in any state or territory) can be moved among states and territories without federal restriction, with the exception of movement to Hawaii. In contrast, regulations at the state and territory levels vary substantially, ranging from no additional regulations beyond those stipulated at the federal level, to strict regulations for the introduction of live colonies, packaged bees, or queens. This variability can lead to inconsistencies in the application of regulations regarding the movement of honey bees among states and territories. In November 2020, we convened a technical working group (TWG), composed of academic and USDA personnel, to review and summarize the (1) history of honey bee importation into/movement within the United States, (2) current regulations regarding honey bee movement and case studies on the application of those regulations, (3) benefits associated with moving honey bees within the United States, (4) risks associated with moving honey bees within the United States, and (5) risk mitigation strategies. This review will be helpful for developing standardized best practices for the safe movement of honey bees between the 48 contiguous states and other states/territories within the United States.

Keywords: honey bee stock, *Apis mellifera*, human mediated movement, regulations, benefits, risks, mitigation strategies

INTRODUCTION

Honey bees, the most common pollinators across plant/pollinator networks worldwide (Hung et al., 2018), are crucial for human food production (Gallai et al., 2009). Western honey bees (*Apis mellifera* Linnaeus, 1758) are the only *Apis* species with a natural distribution that includes Europe, the Middle East, Africa, and parts of western Asia (Meixner et al., 2013). Beekeepers have transported honey bees around the world because they are easy to manage for production purposes (Crane, 1999). *Apis mellifera* now occurs on every continent except Antarctica (Hung et al., 2018) and is the dominant pollinator species used in agriculture (Williams, 1994; Aizen and Harder, 2009; Jordan et al., 2021). Globally, crop pollination and honey production services provided by managed honey bees are valued at $182–577 billion and $8 billion USD/year, respectively (Gallai et al., 2009; Lautenbach et al., 2012; Shahbandeh, 2021).

The human-mediated, global dispersal of western honey bees ultimately led to the development of rules and regulations created to mitigate potential negative outcomes associated with their dispersal. The exportation/importation of honey bees (i.e., transfer of honey bees *between* countries) is regulated at the national level in many countries. These measures range from outright bans on importation to the total absence of regulations. Regulatory authorities in many countries opt for structured permitting processes that, when met, allow the transportation of honey bees, or their germplasm, into a new area under strict protocols monitored by appropriate regulatory authorities. This occurs in the United States under the Honeybee Act, United States Code of Federal Regulation 7 CFR §281–§286 and §322, Subpart B—Importation of Adult Honeybees, Honeybee Germ Plasm, and Bees Other Than Honeybees From Approved Regions (Rules and Regulations, 2014). Regulations regarding the movement of other beneficial bees (i.e., bumble bees, *Bombus* spp.) are also included in this Federal Regulation. Currently, the importation of honey bees into areas where they do not exist is often prohibited worldwide, given concerns of their possible impact on native flora and fauna (Winter et al., 2006; Burgiel and Perrault, 2011).

Regulations concerning the transfer of live honey bees or germplasm *within* an area or political boundary in which they already occur (a process we term "movement" to distinguish from exportation/importation) vary within the United States. In the United States, the states and territories regulate the movement of live honey bees between themselves and other states/territories (Wehling and Flanders, 2005). At the federal level, honey bees already present in the United States (in any state or territory) are allowed to move among states and territories without federal restriction, with the exception of movement to Hawaii (see Code of Federal regulations 7 CFR Parts §319 and §322 at Rules and Regulations, 2014; Wehling and Flanders, 2005; Pernal, 2014). In contrast, regulations at the state and territory levels vary substantially, ranging from no additional regulations beyond the federal ones to strict regulations for the introduction of live colonies, packaged bees, or queens (**Supplementary Table 1**, **Supplementary Figure 1** and interactive map[1]). This variability leads to the inconsistent application of regulations regarding the movement of honey bees among states and territories within the United States.

In November 2020, we convened a technical working group (TWG) formed by academic and USDA personnel, to review and summarize the current status of regulations regarding the human-mediated movement of honey bees within the United States. The TWG was composed of individuals (scientists/staff/graduate students) from the United States Department of Agriculture (USDA) Agriculture Research Service (ARS), the USDA Animal and Plant Health Inspection Service (APHIS), the USDA Farm Production and Conservation (FPAC) Business Center, and Land Grant Universities (see authors and acknowledgment sections). This manuscript represents the collective output of the TWG.

Herein, we review the (1) history of honey bee importation into and movement within the United States, (2) current regulations regarding honey bee movement and case studies on the application of those regulations, (3) benefits associated with moving honey bees within the United States, (4) risks associated with moving honey bees within the United States, and (5) risk mitigation strategies for bees moving within the United States. It is not our intention with this manuscript to suggest policies related to honey bee movement within the United States. We believe that is beyond the scope of this manuscript. Furthermore, we are not proposing a change in current policies regulating movement of honey bee colonies among states/territories under current management scenarios (i.e., for commercial pollination, honey production, or similar existing activities). Instead, we hope this review will be helpful for developing best practices for the safe movement of honey bees between the 48 contiguous states and other states/territories within the United States.

HISTORY OF BEE IMPORTATION INTO/MOVEMENT WITHIN THE UNITED STATES

Historical Importations of Honey Bees Into the United States

Honey bees are not native to the Americas and were imported into the United States as early as 1622 by English settlers (**Supplementary Tables 2**, **3**; Sheppard, 1989a,b). The known

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[1] https://uofmd.maps.arcgis.com/apps/webappviewer/index.html?id=b01d1d7cc19d438d8a6af7ce179a4bca

imported stocks include *A. m. mellifera* Linnaeus, a subspecies with a natural distribution across northern Europe, and *A. m. ligustica* Spinola (Italian honey bee), introduced in the mid-1800's and one of the most favored honey bee stocks (Sheppard, 1989a; Cobey et al., 2012). Other introductions occurred in the 19th century and included *A. m. carnica* Pollmann (Carniolan honey bee), *A. m. caucasica* Pollmann (Caucasian honey bee), *A. m. lamarckii* Cockerell (Egyptian honey bee), *A. m. syriaca* Skorikov (Syrian honey bee), *A. m. cypria* Pollmann (Cyprian honey bee), and *A. m. intermissa* Buttel-Reepen (a north African honey bee). See **Supplementary Tables 2**, **3** for more information.

Introductions of honey bee stocks into the United States over the past several decades are better documented and were done to benefit the beekeeping industry. Queen germplasm from the far-eastern Russian province of Primorsky was introduced into the United States after 7 months of quarantine and rigorous analyses of the biology, behavior, and pest resistance of queens and colonies from 1995 to 2000 (Danka et al., 1995; Rinderer et al., 1997, 1999, 2000, 2001a,b). The bees were reported as gentle, had high honey yields, and maintained low rates of infestation with the mite *Varroa destructor* (Anderson and Trueman, 2000; Rinderer et al., 2001a,b). Russian queen stock was made available to beekeepers in 2001 (Rinderer and Coy, 2020). Currently, a genetic stock certification protocol supports the Russian queen breeding industry (Bourgeois et al., 2020; Rinderer and Coy, 2020). However, this stock is not widely used by beekeepers (Haber et al., 2019).

Historical Movement of Honey Bees Within the United States

Beekeepers readily moved various honey bee stocks throughout the United States after initial stock importation into the country (**Supplementary Table 4**). Furthermore, many colonies swarmed, producing feral colonies that established in many locations and environments. There were no efforts to slow the movement of introduced stock once it was present in the United States. The current United States feral and managed honey bee populations are a genetic mixture of the stocks initially imported into the United States and those subsequently introduced into the United States through the permitting process (Schiff and Sheppard, 1995).

Spread of Honey Bees Into the United States

The introduction of *A. m. scutellata*, a honey bee subspecies of African origin, into Brazil in 1956 greatly impacted the distribution of honey bees in the Americas (Kerr, 1967). Imported into Brazil to increase honey production, 26 colonies of *A. m. scutellata* escaped quarantine in 1957 and hybridized with other *A. mellifera* in the region. These hybrids (Africanized honey bees—AHBs) subsequently spread (e.g., natural dispersal without further human assistance) throughout South and Central America, the southwestern United States (Caron, 2001), and southern Florida (Hall, 1992). Hybridization is well documented across the Americas (Whitfield et al., 2006), and in the United States in particular (Calfee et al., 2020). Africanized honey bees exhibit heightened defensiveness and swarming/absconding behavior and are considered less desirable for commercial use by beekeepers in the United States. Furthermore, AHBs can outcompete and displace honey bees of European descent (EHBs) in areas where they co-occur (Breed et al., 2004; Schneider et al., 2004; Guzman-Novoa et al., 2020).

Africanized honey bee presence in an area does not always result in a negative outcome. This bee has documented resistance to *V. destructor* in Brazil (Carneiro et al., 2007) and in other areas (Strauss et al., 2015; Mondet et al., 2020). Africanized honey bees are generally the preferred honey bee among South and Central American beekeepers who value their honey production and pollination attributes (Roubik and Villanueva-Gutierrez, 2009). Africanized honey bees also occur in Puerto Rico (Puerto Rican Honey Bee or PRHB) where they are notably gentler than the Texas AHB population from which they are derived (Galindo-Cardona et al., 2013; Avalos et al., 2017, 2020; Acevedo-Gonzalez et al., 2019). This gentle demeanor has not been documented in other AHB populations in the Americas. Regulatory authorities in many states within the United States consider the AHB an "unwanted race or stock of honey bee," and thereby prevent its movement into a given state, or its management within the state (**Supplementary Table 1**).

Continued Threat of Honey Bee Introductions Into the United States

The possible accidental introduction of invasive honey bees and their pests/pathogens into the United States is a present and on-going threat (see section "Risks Associated With Moving Honey Bees Among United States and Territories"). APHIS personnel routinely inspect cargo and passengers entering the country for potential insect pest introductions through the Agricultural Quarantine Inspection (AQI) program. APHIS data on intercepts of individuals in the superfamily Apoidea (which includes bees and wasps) from air, maritime, and terrestrial arrivals between 2001 and 2021 show that honey bees (specifically, *A. mellifera*) compose the majority of interceptions and that most honey bee colonies and/or individuals arrive in the United States on plants and stored goods (**Figure 1A**). Furthermore, most of the intercepted honey bees arrive with materials originating from Central and South America. The volume of trade with nations in these regions may account for the number of honey bees intercepted from each country. Apart from *Trypoxylon* spp. (a crabronid wasp genus), most intercepted Apoidea arrived as live adults (**Figure 1B**). These data highlight the continued possibility of accidental or unlawful introductions of honey bees into the United States.

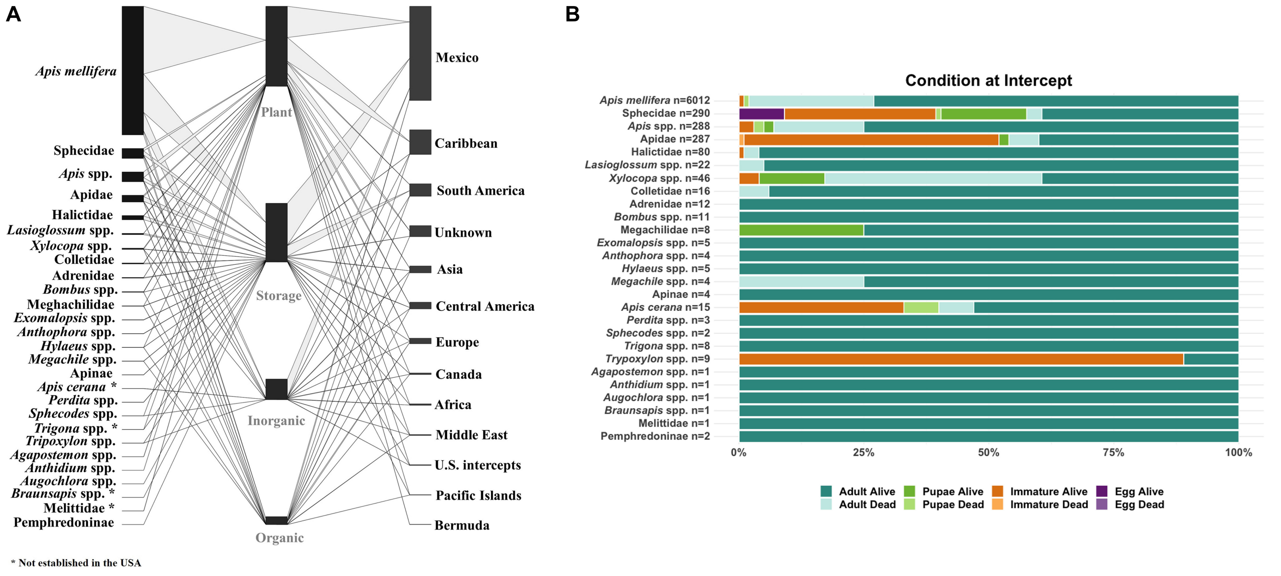

FIGURE 1 | Apoidea intercepts at United States borders (2001–2021), adapted from APHIS's Agricultural Quarantine Activity System (AQAS) data. **(A)** The bee or wasp species (left column) arrived on the material noted (middle column) from the region noted (right column). **(B)** The life stage and condition (dead or alive) of the individual that was found.

UNITED STATES LEGISLATION ON HONEY BEE IMPORTATION AND MOVEMENT

Federal Legislation

The United States Congress enacted the Honeybee Act (Federal Act) on 31 August 1922. Its purpose was to protect honey bees and the beekeeping industry from the tracheal mite, *Acarapis woodi*, the presumed cause of an unknown disease devastating honey bees in Europe at the time (Rennie, 1921). The Act has been promulgated multiple times (presently, 7 USCS §281-286 in effect on 3 January 2012). It has been broadened to regulate the importation of honey bee germplasm into the United States, to contain the spread of harmful diseases and parasites to honey bees within the country, and to eradicate/control undesirable species of *Apis* and subspecies of *A. mellifera* within the country and among imports (Rules and Regulations, 2014). Amendments in 1981 granted the Secretary of Agriculture the ability to approve bee-breeding stock and the release of bee germplasm. The Act now defines "honey bee" as all life stages and germplasm (with the exception of semen) of bees of the genus *Apis* (Rules and Regulations, 2014). As mentioned, there are no federal provisions to regulate honey bees currently present in the United States. This is the result of the perceived lack of risk associated with resident honey bee populations.

State/territory Legislation

Regulations regarding interstate movement of honey bees differ at the state and territory levels within the United States (**Supplementary Table 1**). In general, honey bee movement is regulated by the issuance of permits from the receiving state based on certificates of bee health issued by the shipping state. Interstate movement ranges from no regulatory measures beyond those at the federal level to strict regulations for movement of live colonies, packaged bees, queens, or germplasm. This lack of uniformity has the potential to lead to the rapid spread of introduced pests, pathogens, parasites and/or deleterious honey bee subspecies across the United States, with repercussions for the beekeeping industry (**Box 1**).

BENEFITS OF MOVING HONEY BEES AMONG STATES AND TERRITORIES WITHIN THE UNITED STATES

There are multiple potential benefits to moving honey bees among states and territories within the United States (Russo, 2016). First, migratory beekeeping (i.e., the movement of managed honey bee colonies by beekeepers) has direct value to agriculture because the pollination services provided by bees improves fruit set, crop yield, and quality. Second, moving bees provides direct economic benefit to beekeepers who transport their bees to areas where honey is produced and/or where they are paid to use their colonies to provide crop pollination services. Third, honey bees provide ecological value through the ecosystem service of pollination. Fourth, moving bees supports stock improvement initiatives that require an influx of new genetic material from which to select. Fifth, moving bees can compensate for colony losses at a given location. We provide context for each benefit below, demonstrating that moving bees is necessary within the United States to support a healthy economy, food supply, and beekeeping industry.

BOX 1 | Case study on the spread of honey bee industry threats in the 1980s.

The lack of uniform policies and regulations among states and territories within the United States regarding honey bee movement (**Supplementary Table 1** and **Supplementary Figure 1**) has failed to stop the spread of exotic pests and pathogens introduced to the mainland, with costly and irreversible impacts on the beekeeping industry in the United States. Here, we discuss the spread of honey bee pests and pathogens in the 1980s to illustrate this point, as significant new pests and pathogens established and spread in the United States during this decade.

The United States government passed the Honeybee Act in 1922 in response to the Isle of Wight bee disease in England (Nature, 1912). This act aimed to prevent the importation of honey bees from overseas. In addition, the spread and resulting impact of American foulbrood (bee disease caused by the bacterium *Paenibacillus larvae*) catalyzed the passage of state laws and the implementation of state inspection programs in the 1930s and 1940s (Surface, 1916; Phillips, 1920; Voorhies et al., 1933). In the United States, federal and state governments have distinct roles when protecting agriculture. The federal government, acting through APHIS (United States Department of Agriculture, Animal and Plant Health Inspection Service), attempts to prevent the introduction of honey bee threats into the country, helps develop technologies to detect and monitor potential or introduced threats, and works with state governments to contain newly identified introductions. However, state governments (*via* state departments of agriculture) have regulatory authority once a pest is considered established.

The 1980's Annual Proceedings of the Apiary Inspectors of America reveals the complex and interacting factors that influenced the rate of spread within the United States of three honey bee pests during the 1980s (AIA, 1988d). These pests were honey bee tracheal mites (*Acarapis woodi*, HBTM, arrival date 1984), *Varroa destructor* (formerly *V. jacobsoni*, arrival date 1987) and Africanized honey bees (bees derived from *A.m. scutellata*, AHBs, arrival dates: feral colony intercept at FL ports in 1987 i.e., Panama City, Miami and Fort Lauderdale, followed by colonies found in Hidalgo, TX in 1990). See the timeline in this Box for more information.

For over 50 years since the passage of the Honey Bee Act (1922), the only biological threat to honey bee colonies in the United States was the fungal brood disease chalkbrood (*Ascosphaera apis*). It was first discovered in the United States in 1965, but was likely in the country prior to that time (Aronstein and Murray, 2010). At the same time, the predicted arrival of the AHB to southern states preoccupied lawmakers. The AHB quickly spread north throughout the Americas after its accidental release in Brazil in 1957 (Kerr, 1967). It is notorious for exhibiting heightened defensive responses, compared to those exhibited by *A. mellifera* of Eurasian descent managed by beekeepers in the United States. Identifying this undesirable stock was a difficult and time-consuming process. The inability to identify AHB stock efficiently and with confidence may have played some role in its dispersal. This was also true for bees infested by HBTMs. Proposals to certify stocks or bees as "HBTM free" largely failed because the mites were thought to be disseminated across the country already (AIA, 1987).

When state apiary laws were written, they were not designed to regulate the highly mobile apiary industry of the 1980s. Attempts to restrict the movement of bees, enforce quarantines, or await disease diagnosis were ineffective due to the economic and political demands of modern agriculture, i.e., the need to ship and/or truck bees, queens, and colonies across state lines to ensure the beekeeping industry's viability and human food security through plant pollinated crops. *Varroa destructor*, when first detected on the continent (1987), was already in, or on its way to, several states, and its spread paralleled migratory operations coming out of Florida (ADCP, 1987a). A year after the mites were detected in Florida, they were found in most of the contiguous states (AIA, 1988d). *Varroa destructor* is now ubiquitous across the United States except for the territory of American Samoa and some of the islands of Hawaii, i.e., Maui, Kaua'i, Moloka'i, and Lâna'I (Rusert et al., 2021).

Timeline
Notable Honey Bee Threat Introductions, Response, and Spread in the United States Over the 1980's

1979

- *Varroa destructor* scare, false positive in Maryland (AIA, 1981a).
- *Varroa destructor* response plan developed (AIA, 1981b).

1980

- United States Bee and Honey Act (1980) amended to include regulations to control the importation and interstate movement of diseases and other pests of honey bees (AIA, 1983).
- USDA initiates mite survey in Mexico, discovering the honey bee tracheal mite (*Acarapis woodi*, HBTM, Eischen et al., 1990).

1984

- HBTM first detected in Texas, and subsequently in nine states (AIA, 1985a).
- Florida rescinds all regulations on HBTM (AIA, 1985a). Regulators conclude HBTM cannot be eradicated.
- Consensus that it is best to contain HBTM until control measures are established, but now the pest is widespread, regulations controlling spread may cause more economic hardship than they would alleviate (AIA, 1985c).
- APHIS-PPQ recommends federal laws concerning HBTM be rescinded (AIA, 1985b).
- Nationwide HBTM survey discovering mites only in Florida (AIA, 1985c).

1985

- APHIS deregulates the HBTM (AIA, 1987). They are assumed widespread as migratory beekeepers and package producers continue to ship from Florida (AIA, 1987).

1986

- Each state implements its own protocols for HBTM detection and permitting for the shipment of packages, queens, and movement of colonies (AIA, 1986a).
- Florida queen and package industry "eliminated" by quarantine procedures by other states, halting movement of bees for pollination services and honey production in northern states (AIA, 1986b).

(*Continued*)

Box 1 | (Continued)

1987

- *Varroa destructor* found in Wisconsin, from packaged bees from Florida (ADCP, 1987a).
- *Varroa destructor* in New York State, in migratory beekeeping from Florida (ADCP, 1987a).
- Concluded that restricting migratory movement is impossible to enforce (ADCP, 1987b).
- Canadian border closed to United States queen and package imports, devastating the queen and package industry in the United States (AIA, 1988b).
- Africanized honey bee intercepted at Florida ports (Hall, 1992).

1988

- USDA Action Plans-*Varroa* and Africanized Bees (AIA, 1988c).

1989

- Reaction to USDA action plan largely negative and meets resistance as too restrictive, or not restrictive enough, by different states (AIA, 1988e).

1990

- Feral Africanized honey bee colonies found in mainland United States, at Hidalgo, TX (Visscher et al., 1997).
- *Varroa destructor* arrives in Canada (AIA, 1990).

Benefits of Honey Bee Movement to United States Agriculture

The United States agricultural sector is dependent on insect-mediated pollination for crop yield and/or quality (Jordan et al., 2021). Migratory beekeeping ensures that crop pollination demands are met (Rucker et al., 2012; Ferrier et al., 2018). In the last 50 years, the global demand for pollinator-dependent crops has increased by 300% (Aizen and Harder, 2009; Jordan et al., 2021). The estimated pollination services provided by honey bees to crop production in the United States averages $12–50 billion USD/year (Bauer and Wing, 2010; Calderone, 2012). To preempt pollination deficits, beekeepers move colonies to blooming crops that rely on insect-mediated pollination for fruit, vegetable, and nut production. Those colonies may originate from outside a state if an insufficient number of colonies exists within the state to provide the pollination services needed by the crops grown in that state.

Almond production in California requires a greater number of pollinating colonies than does any other single crop in the United States (Bond et al., 2021). Honey bee pollination of almonds contributed ∼$9.2 billion USD to the California's gross state product (GSP) in the 2017/18 crop year (Matthews et al., 2020), and an estimated direct value of $6.09 billion USD in 2020 (Sumner et al., 2014; NASS, 2020). These are not marginal values; yet they account for a fraction of all marketable services and service fees that honey bees provide through migratory beekeeping. Almonds bloom in California in February, making this crop the starting point of an annual migratory route for many commercial beekeepers (**Figure 2**). Approximately 1.8 million managed honey bee colonies (∼60–75% of all United States commercial colonies) are moved to California each year to provide pollination services for almonds (Perez and Plattner, 2014; Goodrich et al., 2019).

From almonds, beekeepers move these colonies across the country to provide pollination services for additional crops or to major nectar flows around the United States for honey production purposes. Additional colony movements occur during the fall season to allocate hives to warmer latitudes (e.g., Texas, Florida, etc.) for overwintering (Rucker et al., 2012; Jabr, 2013; VPPC, 2016; Bond et al., 2021). Only a subset of beekeepers are migratory. Many provide pollination services only within their region or state.

Commercial Value of Bee Movement for Beekeepers

The movement of honey bees results in direct economic benefit to beekeepers. The revenue generated by providing honey bees for pollination services [rental fees ranging from $70–210 USD per colony, depending on the crop (USDA, 2020)] was estimated at $254.3 million USD in 2020 (NASS, 2021). This value was close to the $299.6 million USD generated from the production of honey in 2020 (NASS, 2021). Wax, pollen, queen, package, and nucleus (nuc) production represent additional value-added products originating from the hive and all generate income for beekeepers.

Ecological Value

Non-marketable ecosystem services are provided *via* the movement of honey bees. These include the improvement of ecosystem function and stability (Losey and Vaughan, 2006). Honey bees are the most frequent visitor of non-crop plants worldwide, being present in about 89% of plant pollination networks (Hung et al., 2018). As a generalist species, honey bees visit a wide variety of flowering plants, while more specialized pollinators only visit a few species or families. Abundant pollination of native and wild vegetation increases the availability of plant resources which, in turn, benefit surrounding wildlife through the production of seeds, berries, nuts, and fruits (Pilati and Prestamburgo, 2016; Klein et al., 2017). In addition, honey bees are often used as bioindicators as they are exposed to trace contaminants such as pesticides and airborne heavy

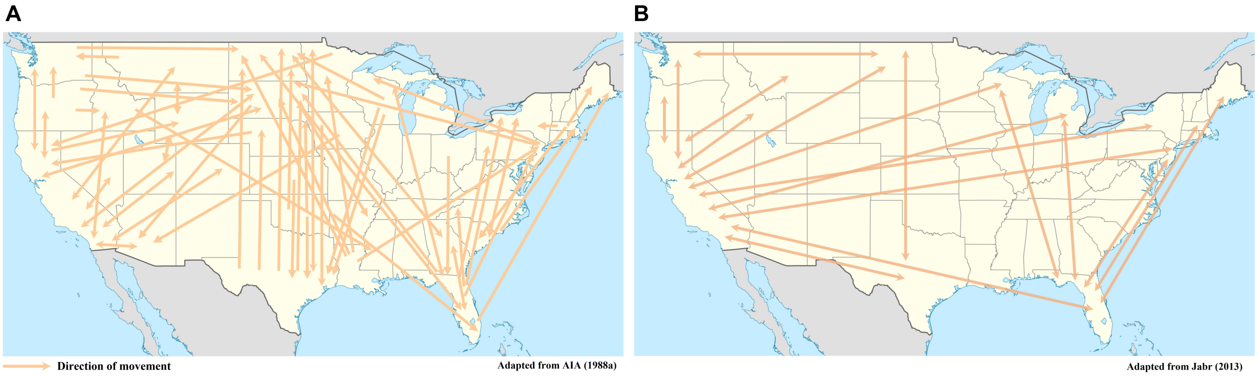

FIGURE 2 | Standard movement of honey bee colonies in migratory beekeeping operations in 1987 **(A)** and 2013 **(B)**. Map lines represent the major movement patterns of honey bee colonies, rather than smaller movements that typically occur within state or contiguous states. Significant movement begins in February (east → west), triggered by California's almond blooming season. Following this, movement occurs mid-March to May (west → northeast) to pollinate apple, pear and cherry orchards, among other crops, and to produce clover honey. A portion of these colonies will be moved to summer locations (northeast → northern plains) to pollinate other crops. During the summer months of June and July, colonies are moved (west coast + south-central → east coast + north) to pollinate apples, cherries, cranberries and vegetables. From mid-August to mid-October, major colony movement occurs (north + northeast →south) for overwintering in warmer latitudes (Rucker et al., 2012; Jabr, 2013; VPPC, 2016; Bond et al., 2021).

metals derived from anthropogenic industrial activities while foraging (Conti and Botrè, 2001; Van der Steen et al., 2011; Kennedy et al., 2013; Pilati and Prestamburgo, 2016; Goretti et al., 2020; Traynor et al., 2021). These contaminants can be traced in nectar/honey, wax, propolis and pollen samples, and can be used as indicators of environmental pollution for a given location (Conti and Botrè, 2001; Finger et al., 2014).

Stock Improvement

The movement of honey bees supports stock improvement programs that advance disease resistance and productivity in a managed stock (Spivak and Reuter, 2001; Traniello et al., 2002; Sadd et al., 2005; Blacquière and Panziera, 2018). Queen breeders and researchers monitor colonies for vigor and health. In response, they can select for traits such as productivity, gentleness and resistance to pests and pathogens (Ferrier et al., 2018). The movement of the selected stocks among states allows beekeepers in other states to incorporate the stocks into their beekeeping operations. As an example, scientists and queen breeders inseminated breeder queens in Hilo, Hawaii with drone germplasm from the USDA *Varroa* sensitive hygiene (VSH) stock (Harbo and Harris, 1999a,b; Ibrahim and Spivak, 2006). Movement of queens from this new stock, the Hilo bee, to the mainland United States is not restricted, but movement of honey bees to Hawaii is regulated.

Disease/pest resistant strains of honey bees can develop as a result of natural selection, making the movement of these bees of potential value to beekeepers. As an example, research suggests that the PRHB provides an opportunity to improve disease and pest resistance in the managed honey bee population in the United States (Rivera-Marchand et al., 2012; Avalos et al., 2017) by their inclusion in selective breeding programs.

Replacement for Colony Losses

Bee movement allows for the replacement of dead colonies. The individual, additive, and synergistic effects of colony stressors are responsible for annual gross colony loss rates averaging 45.5% (Steinhauer et al., 2020; BIP, 2021). Beekeepers mitigate these losses by splitting and/or purchasing colonies or queens of a preferred stock.

RISKS ASSOCIATED WITH MOVING HONEY BEES AMONG STATES AND TERRITORIES WITHIN THE UNITED STATES

There are inherent risks associated with the movement of honey bees among states and territories within the United States. These include the dissemination of honey bee pathogens and pests, spread of resistance genes in a pest/pathogen population, trait dissemination or loss, impacts on other local flora/fauna, and others. It is important to recognize the risks associated with moving honey bees so that appropriate risk mitigation strategies can be developed.

Dissemination of Honey Bee Pests and Pathogens

The movement of a honey bee subspecies or stock risks introducing pests and pathogens that may impact the resident honey bee population (Ji et al., 2003; Moritz et al., 2005; Neumann, 2006; Cavigli et al., 2016; Owen, 2017). The reciprocal is likewise possible, with the introduced stock being naïve to a pest/pathogen endemic in an area, possibly being highly susceptible to it. The United States honey bee population harbors many of the major pests and pathogens known to impact honey bee colonies (Boncristiani et al., 2021), but their distribution and levels differ among states and territories within the United States. Puerto Rico and Hawaii can be used as examples of this difference. Deformed Wing Virus-A (DWV-A) and Acute Bee Paralysis Virus (ABPV) are the most prevalent honey bee viruses in Puerto Rico (APHIS, 2018), while Chronic Bee Paralysis Virus (CBPV), Israeli Acute Paralysis Virus (IAPV), and Slow Bee Paralysis Virus (SBPV) have not been detected there (APHIS, 2018). The movement of honey bees between the continental United States and Puerto Rico could result in the spread of these viruses from the continent to managed honey bees on the island (Grozinger and Flenniken, 2019). In Hawaii, *V. destructor* occurs in honey bee colonies on some islands but not on others (Ramadan et al., 2019; Beaurepaire et al., 2020; Boncristiani et al., 2021; Rusert et al., 2021). Moving bees among islands could spread *V. destructor* to islands where it does not occur.

Some honey bee stocks may harbor new pests/pathogens not broadly distributed in the United States. For example, an in-depth genetic analysis of United States mainland and Hawaiian honey bee samples collected during the 2015 APHIS National Honey Bee Pest and Diseases Survey found new viruses not linked to any reported clinical signs (Ray et al., 2020). The detection of these new viruses raises concerns that other undetected pests and pathogens exist, leading to their possible transmission when honey bee colonies are moved.

Honey bee movement may lead to the future dissemination of organisms likely to prove harmful to the beekeeping industry (see **Box 1**). These include *Tropilaelaps* spp., a genus of mites with members that are pests of honey bees in Asia (Anderson and Morgan, 2007), and *Nosema neumanni*, a new species of *Nosema* recently discovered in Africa (Chemurot et al., 2017). This list also includes *A. mellifera* species of concern, such as *A. m. capensis* (the Cape honey bee), known to be social parasite of managed honey bee colonies in South Africa (Neumann and Moritz, 2002).

Spread of Resistance Genes in a Pest/pathogen Population

Honey bee movement can result in the spread of resistance genes in pest/pathogen populations. As an example, some populations of *V. destructor* have documented resistance to amitraz, fluvalinate, and coumaphos in different areas within the United States (Kast et al., 2020; Rinkevich, 2020; Millán-Leiva et al., 2021). Correspondingly, moving honey bees that host miticide-resistant *V. destructor* could spread the resistance trait to miticide-susceptible *V. destructor* populations (Benito-Murcia et al., 2021).

Trait Dissemination or Loss

The movement of honey bees could lead to the spread of negative traits from the introduced population to the resident managed population and *vice versa*. For example, introduced AHBs express heightened defensive behavior, absconding tendencies, etc. and these can be incorporated, *via* hybridization, into the managed honey bee population in areas where AHBs spread (Caron, 2001; Schneider et al., 2004).

Bee movement can impact heritable traits in other ways as well. A selected stock that exhibits beneficial traits can lose those traits when moved into a new area. This occurs because of open mating, i.e., newly produced queens leaving their hives to mate with multiple drones from different colonies among the local population (Koeniger et al., 2014). As an example, selected breeding stock could lose some of its desirable traits, e.g., gentleness and resistance to *V. destructor*, when moved into an area with established honey bee colonies. This may occur even when the original intent of the proposed move was to introduce selected traits into resident managed populations.

Honey bees (resident or introduced) may lose or lack adaptations that improve their health, productivity, and survival in a specific area. Multiple research teams have demonstrated that honey bees in a selection program can fail to perform as expected when moved to a different environment with different climatic conditions (Costa et al., 2012; Hatjina et al., 2014; Kovaèiæ et al., 2020). For example, a pan-European evaluation on behavioral traits across 16 genotypes of five honey bee subspecies in various environmental conditions across Europe showed that genotype and location can impact traits such as defensiveness and swarming behavior (Uzunov et al., 2014). The widespread movement of a large percentage of the managed honey bee population in the United States may limit the development of locally adapted honey bee stocks.

Impacts on Other Local Flora/Fauna

Honey bee colonies, shipped packages, and bees may harbor other arthropods, pests, and pathogens that can impact local bees, other animals, and plant communities (Graystock et al., 2016). Furthermore, plant communities can change when honey bees are moved into an area. This occurs when honey bees pollinate a variety of flowering species, resulting in increased weed species (Goulson and Derwent, 2004) or reduced ranges of range-restricted plants (Norfolk et al., 2018). Moved honey bees may also impact local pollinator communities through competition and displacement (Torné-Noguera et al., 2016; Cane and Tepedino, 2017). The placement of honey bee colonies in high densities at a given location reduces the connectedness of plant-pollinator networks, and in some cases may lead to a decline in ecosystem resilience (Goulson, 2003; Guimarães, Jordano and Thompson, 2011; Geslin et al., 2017; Valido et al., 2019). Additionally, there is evidence for population-level changes with native bee and flora decline in areas where honey bees are the dominant pollinator. This results in the homogenization of honey bee pollinated fauna (Thomson, 2004, 2016; Paini and Roberts, 2005; McKinney and La Sorte, 2007; Balfour et al., 2015; Goulson et al., 2015; Guzman-Novoa et al., 2020).

It is noteworthy that most of these studies document the potential for large-scale impacts on resident native pollinator populations from introduced non-native bees such as honey bees, and not empirical evidence of direct effects on native bee fitness, abundance, and diversity in response to honey bee competition. Usually, negative impacts are predicated on the assumption that natural communities are at capacity and all plants are pollinated efficiently. However, this is not always the case as has been shown in neotropical communities (Ackerman, 2021). Investigators, through long-term studies on the impact of AHBs on native solitary bees in Central and South America, failed to identify impacts of AHB introductions on native orchid bees. In fact, they found that orchid bee populations increased in numbers after AHBs became established. This finding may be explained by increased foraging resources resulting from pollinator services provided by honey bees (Roubik, 2009; Roubik and Villanueva-Gutierrez, 2009). If honey bees do negatively impact non-*Apis* bee communities in the United States, it is likely to be concentrated around apiaries, and the roadways that give beekeepers access to them, and influenced by local environmental characteristics. The magnitude of honey bee impacts on native pollinators and plant communities depends on local parameters such as foraging bloom density and patchiness, distance from forage, hive density and host specificity of the native pollinators (Cane and Tepedino, 2017; Mallinger et al., 2017).

Other Risks

Every year, beekeepers move more than 1.8 million honey bee colonies around the United States to provide good forage sources for their bees, manage them in more favorable climates/environments, and gain profit by making honey and/or providing pollination services (Ferrier et al., 2018; Goodrich et al., 2019; Bond et al., 2021). There is always a risk of accidents when moving bees on large trucks that typically hold *ca.* 400 colonies. An overturned trailer may contain 1.2 million or more bees and they pose significant risk to people involved in the accident, those passing it, and the first responders addressing it. Sensationalized press coverage of such events, including AHBs or "killer" bees as often called by the press, can harm the reputation of the industry, and may make the public more cautious about bees and beekeepers.

Transporting bees can harm them directly. Repetitive relocation of bees during migratory beekeeping seasons can induce oxidative stress, lead to overheating, and decrease the lifespan of a colony (Ahn et al., 2012; Simone-Finstrom et al., 2016). Multiple relocations, and travel itself, also leads to higher exposure to pesticides and other agrochemicals, which can affect colony survival (Mullin et al., 2010; vanEngelsdorp and Meixner, 2010; Doublet et al., 2015). In addition, high colony densities on moving trucks can lead to pest and pathogen transmission and intensify disease outbreaks. A stress response is also triggered by the need for foraging bees to reassess their changing environment continuously (Simone-Finstrom et al., 2016). vanEngelsdorp et al. (2012) inspected colonies in three migratory beekeeping operations in the eastern United States, quantifying survivorship and prevalence of pests and pathogens. The team identified two risk factors that were predictive of colony mortality in migratory operations, idiopathic brood disease syndrome (IBDS), where brood of different ages appears molten on the bottom of their cells, and queen replacement or failure. In addition, Zhu et al. (2014) reported that transportation increases the abundance and prevalence of *N. ceranae* in honey bees. Moving bees to better forage may counter these effects and ultimately improve the growth and survival of colonies.

RISK MITIGATION STRATEGIES FOR HONEY BEE MOVEMENT AMONG STATES AND TERRITORIES WITHIN THE UNITED STATES

There are means to mitigate the risks associated with moving honey bees. A successful risk mitigation approach integrates multiple risk reduction strategies. Here, we review risk mitigation strategies associated with bee movement and discuss their feasibility of adoption, which may depend on individual state regulations already in place (**Supplementary Table 1**).

Timely Detection of Pests, Pathogens, and Negative Behavioral Traits

An important risk mitigation strategy could include screening the honey bee stock/life stage (i.e., adult bees, immature bees, and germplasm) proposed for movement between the contiguous 48 states and outlying states/territories for pests and pathogens they may harbor, or undesirable behavioral traits they may display. The APHIS National Honey Bee Pests and Diseases Survey has expanded to screen for additional agents of concern (Traynor et al., 2016; Fahey et al., 2018, 2019; Ray et al., 2020). These types of surveys can target the detection of specific pathogens or pests present in the country (e.g., *V. destructor*, *Nosema* spp., etc.), or of major concern if they were introduced (e.g., *Tropilaelaps* spp., *N. neumanni,* SBPV).

High-throughput sequencing and additional molecular technologies can detect viruses and other microbes infecting honey bees (Cox-Foster et al., 2007). Novel and inexpensive metagenomic screening tools exist and are used to detect viruses in bee communities (Galbraith et al., 2018). These are also used for detection of viruses in honey bees (Runckel et al., 2011; Granberg et al., 2013; Beaurepaire et al., 2020). Environmental DNA (eDNA) has been used to screen for pathogens and pests in honey (Ribani et al., 2020), while shotgun sequencing of honey can identify traces of organisms that bees encounter while foraging (Bovo et al., 2020).

There are also techniques for screening for unwanted species/subspecies of honey bees, though they vary in degree of accuracy. For example, the African honey bee, *A. m. scutellata*, and its hybrids can be identified using a reduced set of single nucleotide polymorphisms (SNPs), a real-time qPCR assay, or combinations of morphological features (Pinto et al., 2014; Harpur et al., 2015; Munoz et al., 2015; Eimanifar et al., 2018, 2020; Boardman et al., 2021; Momeni et al., 2021). Geo-morphometric analyses of honey bee wings coupled with SNP data (Calfee et al., 2020; Henriques et al., 2020), or geo-morphometrics alone (Nawrocka et al., 2018; Bustamante et al., 2020) have been used to identify *A. m. scutellata* populations as well. However, there are problems with relying on the output from some of these screening techniques. For example, morphometric methods can fail to assign hybrid populations accurately (Guzman-Novoa et al., 1994). Furthermore, methods that rely on mitochondrial DNA are limited as they do not account for paternal contributions (Meusel and Moritz, 1993).

Colonies can also be screened for visible signs of infection/infestation by pathogens/pests and for the presence of undesirable traits using standard protocols (e.g., Shimanuki and Knox, 2000; Dietemann et al., 2013; Spivak and Reuter, 2016). Some behavioral traits could be screened for using molecular technologies (Avalos et al., 2020) or in the field (Giray et al., 2000; Avalos et al., 2014). There are caveats to these trait screening tools when genes governing a phenotype are not yet known, making a molecular screen impossible, or when a phenotype does not manifest in colony-based assays, as in the case of heightened absconding behavior.

A comprehensive monitoring program that profiled the microbiome and behavioral traits of honey bee stocks proposed for movement would be beneficial on a regional/country wide level. These activities could be considered as an integral component of the decision-making process for the inter-regional movement of honey bee stock to a new area.

Phased Movement Within United States Land

Phased movement includes the movement of a honey bee stock into an area after being quarantined. Phased imports serve as an example of how phased movement within the United States could be implemented. APHIS developed procedures for phased imports to mitigate the spread of pests or pathogens from foreign stock to the United States. Similar procedures could be implemented when proposing the movement of honey bees from United States island territories to the mainland and *vice versa*. Here, we provide two examples of phased imports, the first for honey bee queens and the second for germplasm.

USDA scientists implemented a phased import strategy for the Russian honey bee (or Primorsky bee). Briefly, they imported 100 Russian honey bee queens and quarantined them on Grand Terre Island, USDA-ARS Honey Bee Quarantine Station, Louisiana, United States in 1997. The scientists re-queened colonies of local stock with the Russian queens and monitored the colonies for pest and disease resistance, behavior, offspring vigor and resistance to *V. destructor*. The quarantine lasted seven months until the new stock was certified free of notifiable pests and pathogens (Rinderer et al., 1999). A Russian queen commercialization and stock certification protocol is now established in the United States *via* the Russian Honey Bee Breeders Association (Bourgeois et al., 2020; Rinderer and Coy, 2020).

Phased movements of germplasm can follow the strategy implemented for phased imports of germplasm, which include the importation of germplasm into a quarantined stock before its release. Sheppard (2012) documented the importation of honey bee semen from managed stocks of *A. m. ligustica* (from Bologna, Italy, 2008, 2009*), A. m. carnica* (from Kirchhain, Germany, 2008, 2009) and *A. m. caucasica* (from the Tblisi and other locations, Republic of Georgia, 2010) to quarantine sites located in the wheat growing Palouse region of eastern Washington (Bald Butte and Smoot Hill, Whitman County). The imported semen was used to inseminate unmated queens for stock improvement purposes in the United States (Sheppard, 2012). The wheat fields consisted of several hundred hectares with restricted access and were surrounded by hectares of crops that do not provide resources to honey bees (e.g., lentils, barley, etc.). This experimental plan minimized interactions between the research stock and colonies managed by beekeepers in the area. The semen was screened for viruses prior to its use for the insemination of clipped queens. The queens were maintained in nucleus colonies under quarantine until approval was received from APHIS for their release.

Regulate Interstate and Territory Movement

Another mitigation strategy involves regulating interstate/territory movement following a standard protocol, similar to the one used at the national level for stock importation (Regulation 7 C.F.R. §322 at Rules and Regulations, 2014). In this case, bee movement is permitted when the honey bee stock or germplasm is accompanied by a permit or certificate that states the bees are free of pests, pathogens, and/or harmful phenotypes. This would trigger federal and state reviews of the risks and benefits of moving the bees. Such protocols exist but are not applied consistently across states (**Supplementary Table 1**).

Admittedly, regulating movement at the state level through inspections, certifications, and permits can be arduous and time-consuming. However, a vetted and implemented permitting process, triggered by notifying APHIS personnel with a request to move bees, could remove some of the inconsistencies between state and territory regulations regarding the movement of honey bees (Mailander and Grant, 2019). The adoption of a standardized permitting process across all states and territories may translate into a more uniform and judicious regulation of bee stock movement.

Instrumental Insemination and the Introduction of Germplasm Into an Existing Honey Bee Population

The movement of germplasm, rather than actual bees, can mitigate some risks associated with moving honey bees. Safeguards can be implemented to ensure pest-free germplasm. The movement of drone semen, for example, does not carry the risk of moving *Varroa* spp., *Tropilaelaps* spp., or other arthropod pests. Semen can be screened for pathogens using molecular tools and discarded at low cost if pathogens are discovered (Sheppard, 2012). Furthermore, germplasm is easier to transport, does not carry the risk of bee escape, and can be introduced into resident populations of honey bees through instrumental insemination (Hopkins and Herr, 2010; Hopkins et al., 2012; Sheppard, 2012). Due to these advantages, it was the protocol chosen for the introduction of Russian honey bees, *A. m. ligustica*, *A. m. carnica*, and *A. m. caucasica* germplasm into the United States (Sheppard, 2012). This method was also used for the Hilo and Pol-line stocks kept in Hawaii in closed mating systems for queen releases to commercial beekeepers (Danka et al., 2016; Bilodeau et al., 2020).

Stock Certification Program

A stock certification program can include multiple risk mitigation strategies. First, a stock can be defined per its attributes, such as possessing a selected trait (i.e., *Varroa* Sensitive Hygiene) or combination of traits that make is resistant to a pest or pathogen. The latter happened in the case of the Russian honey bee, with its origin and general resistance to *V. destructor* being integral to the stock's defined attributes (Bourgeois et al., 2020). Second, a certification program can include routine screening for pests, pathogens, and undesirable traits. If the stock is derived from an "unwanted race or subspecies" of honey bee (language often used at the state regulatory level), a demonstration that the stock is free of the "unwanted" trait could be included as part of the stock's definition.

It is important to demonstrate that the genetic stock will not re-acquire, or revert to, undesirable phenotypes. It is also necessary to develop a maintenance plan for the stock. This could include a strategy to maintain and monitor the stock's purity, along with a demonstration that it is not prone to broad geographic dissemination and competition with resident populations. The classic example of a failure to contain invasive bees is the introduction of the AHB into Brazil in 1956 (Kerr, 1967). Multiple AHB queens escaped from research colonies before adequate safeguards were put in place, leading to the establishment of one of the most successful invasive insects in the Americas (Smith, 1991; Visscher et al., 1997; Caron, 2001; Moritz et al., 2005).

Prohibit Movement of Honey Bee Stock Into an Area in Which It Is Not Already Present

Another risk mitigation strategy involves the prohibition of movement of honey bee stocks to areas they are not present. This represents the most extreme mitigation strategy and is employed only if the risk is so great that a negative outcome is almost ensured. It is necessary to ensure that a prohibition of this type would not affect the normal movement of honey bees for typical beekeeping purposes. In most cases, this mitigation strategy is probably not practical.

Additional Considerations

There are additional variables to consider when developing appropriate mitigation strategies. First, the level of mitigation can vary depending on what is being moved (i.e., queens, packages, colonies, germplasm, etc.). Second, certification requirements for a given move request would depend on its specific characteristics (e.g., island setting vs. mainland setting, variable regulatory frameworks between states, etc.). Third, a testing framework and appropriate certification process would be required for genetic, pest, and pathogen testing that ascribes to specific standards, such as the Good Laboratory Practices (GLP). Adherence to accepted standards could be demonstrated by private and federal laboratories that may conduct such tests. Fourth, it is important to consider the regulatory costs for monitoring movement. Consistency on movement policies and risk mitigation strategies at federal and state levels is needed for a country-wide regulatory framework to function in an efficient manner. The serious consideration of the issues presented in this manuscript can serve as the basis upon which to build a framework that will lead to a fair, economic, equitable and beneficial process for moving honey bees between states and territories within the United States, especially between the 48 contiguous states and the outlying states/territories.

GENERAL CONCLUSION

Honey bees are vitally important to agriculture in the United States, ensuring a significant portion of the nation's food supply through the pollination services they provide. Beekeepers move colonies to provide pollination services, produce honey, recover colony losses, improve stocks *via* new genetic material, and recuperate costs. The movement of honey bees is a necessary and critical component of agriculture in the United States and it supports the beekeepers whose incomes rely on healthy bees. Although necessary, the movement of honey bees is not without risk. This risk has led state and territory officials to develop a variety of rules and regulations imposed to ensure honey bee health and sustainability of the beekeeping industry.

The issues associated with the regulatory process and regulations regarding the movement of honey bees among states and territories within the United States led to this review. We believe bee movement remains a necessary and critical component of agriculture and the beekeeping industry in the United States. The information we present herein can inform future efforts designed to create standardized best practices for moving honey bees among states and territories within the United States.

AUTHOR CONTRIBUTIONS

JM and JEl conceived and developed the review. JEv, ToG, TuG, EN, RR, WS, and DvE provided empirical knowledge and scientific expertise regarding the application of current state and federal regulations on bee movement, present status of the bee industry and beekeeping in states and territories within the United States, and risks and mitigation strategies associated with moving honey bees within the United States. KC, AN, and CB performed data analysis, figure preparation, and interactive map assembly. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.850600/full#supplementary-material

REFERENCES

Acevedo-Gonzalez, J. P., Galindo-Cardona, A., Avalos, A., Whitfield, C. W., Rodriguez, D. M., Uribe-Rubio, J. L., et al. (2019). Colonization history and population differentiation of the Honey Bees (*Apis mellifera* L.) in Puerto Rico. *Ecol. Evol.* 9, 10895–10902. doi: 10.1002/ece3.5330

Ackerman, J. D. (2021). Island invasions by introduced honey bees: what can be expected for Puerto Rico and the Caribbean? *Front. Ecol. Evol.* 10:556744. doi: 10.3389/fevo.2020.556744

ADCP (1987a). *Proceedings of Apiary Disease Control Program, New York State 1987*. New York, NY: Apiary Disease Control Program, 5–7. *Varroa mites* in New York State.

ADCP (1987b). *Proceedings of Apiary Disease Control Program, New York State 1987*. New York, NY: Apiary Disease Control Program, 294–225. Apiary advisory committee recommendations: Tracheal mites.

Ahn, K., Xie, X., Riddle, J., Pettis, J., and Huang, Z. Y. (2012). Effects of long-distance transportation on honey bee physiology. *Psyche* 2012:193029. doi: 10.1007/s00359-002-0374-y

AIA (1981a). "Proceedings of the Annual Conference Apiary Inspectors of America 1981," in *Annual Conference Apiary Inspectors of America*, ed. I.Barton Smith Jr. (Las Vegas, NV), 26–28. *Varroa* scare in Maryland.

AIA (1981b). "Proceedings of the Annual Conference Apiary Inspectors of America 1981, January 12-14," in *Recommendations to the beekeeping industry concerning the mite Varroa jacobsoni. Annual Conference Apiary Inspectors of America*, Las Vegas, NV, 28–30.

AIA (1983). "Proceedings of the Annual Conference Apiary Inspectors of America 1983, January," in *Amendments to the United States Bee and Honey Act Annual Conference Apiary Apiary Inspectors of America Orlando*, Florida, 46.

AIA (1985a). "Proceedings of the Annual Conference Apiary Inspectors of America 1985, January 21-25," in *Federal Survey and Control Relating to African Bee, Varroa and Acarapis Mites by Mr. Richard L. Cowden, Senior Staff Officer, National Program Planning Staff, USDA Annual Conference Apiary Inspectors of America San Antonio*, Texas, 33–34.

AIA (1985b). "Proceedings of the Annual Conference Apiary Inspectors of America 1985, January 21-25," in *Current status of honey bee tracheal mite, USDA-APHIS. Annual Conference Apiary Inspectors of America, San Antonio*, Texas, 35–37.

AIA (1985c). "Proceedings of the Annual Conference Apiary Inspectors of America 1985 January 21-25," in *Technical Advisory committee for Africanized Bees and Honey Bee Mites Survey results. Annual Conference Apiary Inspectors of America San Antonio*, Texas, 38–42.

AIA (1986a). "Proceedings of the Annual Conference Apiary Inspectors of America 1986, January 27-30," in *Tracheal mite Certification of Honey bees Annual Conference Apiary Inspectors of America Baton Rouge*, Louisiana, 48–49.

AIA (1986b). "Proceedings of the Annual Conference Apiary Inspectors of America 1986, January 27-30," in *Beekeeping in Florida by Laurence P. Cutts. Annual Conference Apiary Inspectors of America Baton Rouge*, Louisiana, 66–70.

AIA (1987). "Proceedings of the Annual Conference Apiary Inspectors of America 1987, January 26-29," in *Politics of Tracheal Mites by Dr. H. Shiminuki, Laboratory Director USDA, Beltsville MD. Annual Conference Apiary Inspectors of America*, Biloxi, MS, 19–21.

AIA (1988a). "Interstate migratory beekeeper and colony survey," in *Proceedings Annual Conference of Apiary Inspectors of America. 1988 October 10-14*, Denver, CO, 49–58.

AIA (1988b). "Proceedings of the Annual Conference Apiary Inspectors of America 1988, January 18-21," in *CAPA report By Mr. Will Kissinger. Annual Conference Apiary Inspectors of America*, Baltimore, MD, 47.

AIA (1988c). "Proceedings of the Annual Conference Apiary Inspectors of America 1988, January 18-21," in *USDA Action Plans-Varroa and Africanized bees Annual Conference Apiary Inspectors of America*, Baltimore, MD, 24.

AIA (1988d). "Proceedings of Fall Annual Conference Apiary Inspectors of America 1988, October 10-14," in *AIA General session Proceedings Annual Conference of Apiary Inspectors of America*, Baltimore, MD, 16.

AIA (1988e). "Proceedings of Fall Annual Conference Apiary Inspectors of America 1988, October 10-14," in *Summary of Proposed Varroa rules responses by AIA (May1989) Washington State Department of Agriculture Proceedings Annual Conference of Apiary Inspectors of America*, Denver, CO, 69–80.

AIA (1990). "Proceedings of the Annual Conference Apiary Inspectors of America 1990, January 12," in *CAPA report By Doug McRory Annual Conference Apiary Inspectors of America*, Schaumburg, IL, 26.

Aizen, M. A., and Harder, L. D. (2009). The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. *Curr. Biol.* 19, 915–918. doi: 10.1016/j.cub.2009.03.071

Anderson, D. L., and Trueman, J. W. H. (2000). *Varroa jacobsoni* (Acari: Varroidae) is more than one species. *Exp. App. Acarol.* 24, 165–189. doi: 10.1023/a:1006456720416

Anderson, Dl, and Morgan, M. J. (2007). Genetic and morphological variation of bee-parasitic *Tropilaelaps mites* (Acari: Laelapidae): new and re-defined species. *Exp. Appl. Acarol.* 43, 1–24. doi: 10.1007/s10493-007-9103-0

APHIS (2018). *State Report, Puerto Rico. Bee Informed Partnership*. Available online at https://bip2.beeinformed.org/reports/state_reports/state_report/?year=2018&state=PR (accessed April 26, 2022).

Aronstein, K. A., and Murray, K. D. (2010). Chalkbrood disease in honey bees. *J. Invertebr. Pathol.* 103, S20–S29.

Avalos, A., Fang, M., Pan, H., Lluch, A. R., Lipka, A. E., Zhao, S. D., et al. (2020). Genomic regions influencing aggressive behavior in honey bees are defined by colony allele frequencies. *Proc. Natl. Acad. Sci. U.S.A.* 117, 17135–17141. doi: 10.1073/pnas.1922927117

Avalos, A., Rodriguez-Cruz, Y., and Giray, T. (2014). Individual responsiveness to shock and colony-level aggression in honey bees: evidence for a genetic component. *Behav. Ecol. Sociobiol.* 68, 761–771. doi: 10.1007/s00265-014-1689-8

Avalos, H., Pan, C., Li, J. P., Acevedo-Gonzalez, G., Rendon, C. J., Fields, P. J., et al. (2017). A soft selective sweep during rapid evolution of gentle behavior in an Africanized honey bee. *Nat. Commun.* 8:1550. doi: 10.1038/s41467-017-01800-0

Balfour, N. J., Gandy, S., and Ratnieks, F. L. W. (2015). Exploitative competition alters bee foraging and flower choice. *Bahav. Ecol. Sociobiol.* 69, 1731–1738. doi: 10.1007/s00442-019-04576-w

Bauer, D. M., and Wing, I. S. (2010). Economic consequences of pollinator declines: a synthesis. *Agric. Resour. Econ. Rev.* 39, 368–383. doi: 10.1017/s1068280500007371

Beaurepaire, A., Piot, N., Doublet, V., Antunez, K., Campbell, E., and Chantawannakul, P. (2020). Diversity and global distribution of viruses of the western honey bee. *Apis mellifera*. *Insects* 11:239. doi: 10.3390/insects11040239

Benito-Murcia, M., Bartolomé, C., Maside, X., Bernal, J., Bernal, J. L., del Nozal, M. J., et al. (2021). Residual tau-fluvalinate in honey bee colonies is coupled with evidence for selection for *Varroa destructor* resistance to pyrethroids. *Insects* 12:731. doi: 10.3390/insects12080731

Bilodeau, L., Avalos, A., and Danka, R. G. (2020). Genetic diversity of the complementary sex-determiner (csd) gene in two closed breeding stocks of *Varroa*-resistant honey bees. *Apidologie* 51, 1125–1132. doi: 10.1007/s13592-020-00790-1

BIP (2021). *Total Winter All Colony. Bee Informed Partnership*. Available online at: https://beeinformed.org/2021/06/21/united-states-honey-bee-colony-losses-2020-2021-preliminary-results/ (accessed April 26, 2022).

Blacquière, T., and Panziera, D. (2018). A plea for use of honey bees' natural resilience in beekeeping. *Bee World* 95, 34–38. doi: 10.1080/0005772X.2018.1430999

Boardman, L., Srivastava, P., Jeyaprakash, A., Moore, M. R., Whilby, L., and Ellis, J. D. (2021). A qPCR assay for sensitive and rapid detection of African A-lineage honey bees (*Apis mellifera*). *Apidologie* 52, 767–781. doi: 10.1007/s13592-021-00863-9

Boncristiani, H., Ellis, J. D., Bustamante, T., Graham, J., Jack, C., and Kimmel, C. B. (2021). World honey bee health: the global distribution of western honey bee (*Apis mellifera* L.) pests and pathogens. *Bee World* 98, 2–6. doi: 10.1080/0005772x.2020.1800330

Bond, J. K., Hitaj, C., Smith, D., Hunt, K., Perez, A., and Ferreira, G. (2021). *Honey Bees on the Move: From Pollination to Honey Production and Back. ERR-290*. Washington, DC: U.S. Department of Agriculture, Economic Research Service.

Bourgeois, L., Sheppard, W. S., Sylvester, H. A., and Rinderer, T. E. (2020). Genetic stock identification of russian honey bees. *J. Econ. Entomol.* 103, 917–924. doi: 10.1603/ec09335

Bovo, S., Utzeri, V. J., Ribani, A., Cabbri, R., and Fontanesi, L. (2020). Shotgun sequencing of honey DNA can describe honey bee derived environmental signatures and the honey bee hologenome complexity. *Sci. Rep.* 10:9279. doi: 10.1038/s41598-020-66127-1

Breed, M. D., Guzmán-Novoa, E., and Hunt, G. J. (2004). Defensive behavior of honey bees: organization, genetics, and comparisons with other bees. *Ann. Rev. Entomol* 49, 271–298. doi: 10.1146/annurev.ento.49.061802.123155

Burgiel, S. W., and Perrault, A. M. (2011). "Black, white, and gray lists," in *Encyclopedia Of Biological Invasions*, eds M. Rejmánek and D. Simberloff (Berkeley: University of California Press), 75–77. doi: 10.1525/9780520948433-021

Bustamante, T., Baiser, B., and Ellis, J. D. (2020). Comparing classical and geometric morphometric methods to discriminate between the South African honey bee subspecies *Apis mellifera scutellata* and *Apis mellifera capensis* (Hymenoptera: Apidae). *Apidologie* 51, 123–136. doi: 10.1007/s13592-019-00651-6

Calderone, N. (2012). Insect pollinated crops, insect pollinators and US agriculture: trend analysis of aggregate data for the period 1992–2009. *PLoS One* 7:e37235. doi: 10.1371/journal.pone.0037235

Calfee, E., Agra, M. N., Palacio, M. A., Ramirez, S. R., and Coop, G. (2020). Selection and hybridization shaped the rapid spread of African honey bee ancestry in the Americas. *PLoS Genet.* 16:e1009038. doi: 10.1371/journal.pgen.1009038

Cane, J. H., and Tepedino, V. J. (2017). Gauging the effect of honey bee pollen collection on native bee communities. *Conserv. Lett.* 10, 205–210. doi: 10.1111/conl.12263

Carneiro, F. E., Torres, R. R., Strapazzon, R., Ramírez, S. A., Guerra, J. C. V. Jr., Koling, D. F., et al. (2007). Changes in the reproductive ability of the *Varroa destructor* (Anderson & Trueman) in Africanized honey bees (*Apis mellifera* L.) (Hymenoptera: Apidae) colonies in southern Brazil. *Neotropical. Entomol.* 36, 949–952. doi: 10.1590/s1519-566x2007000600018

Caron, D. M. (2001). *Africanized Honey bees in the Americas*. Medina, OH: The A.I. Root Co, 228.

Cavigli, I., Daughenbaugh, K. F., Martin, M., Lerch, M., Banner, K., Garcia, E., et al. (2016). Pathogen prevalence and abundance in honey bee colonies involved in almond pollination. *Apidologie* 47, 251–266. doi: 10.1007/s13592-015-0395-5

Chemurot, M., De Smet, L., Brunain, M., De Rycke, R., and de Graaf, D. C. (2017). *Nosema neumanni* n. sp. (Microsporidia, Nosematidae), a new microsporidian parasite of honeybees, *Apis mellifera* in Uganda. *Eur. J. Protistol.* 61(Pt A), 13–19. doi: 10.1016/j.ejop.2017.07.002

Cobey, S. W., Sheppard, W. S., and Tarpy, D. R. (2012). "Status of breeding practices and genetic diversity in domestic us honey bees," in *Honey Bee Colony Health*, eds D. Sammataro and J. A. Yoder (Boca Raton, FL: CRC Press. Taylor and Francis).

Conti, M. E., and Botrè, F. (2001). Honeybees and their products as potential bioindicators of heavy metals contamination. *Environ. Monit. Assessment* 69, 267–282. doi: 10.1023/a:1010719107006

Costa, C., Büchler, R., Berg, S., Bienkowska, M., Bouga, M., Bubalo, D., et al. (2012). A Europe-wide experiment for assessing the impact of genotype–environment interactions on the vitality and performance of honey bee colonies: experimental design and trait evaluation. *J. Apic. Sci.* 56, 147–158. doi: 10.2478/v10289-012-0015-9

Cox-Foster, D. L., Conlan, S., Holmes, E. C., Palacios, G., Evans, J. D., Moran, N. A., et al. (2007). A metagenomic survey of microbes in honey bee colony collapse disorder. *Science* 318, 283–287. doi: 10.1126/science.1146498

Crane, E. (1999). *The World History of Beekeeping and Honey Hunting*. Abingdon: Taylor and Francis Group, 704.

Danka, R. G., Dodds, G. E., and Harris, J. W. (2016). Selection of VSH-derived "Pol-line" honey bees and evaluation of their *Varroa*-resistance characteristics. *Apidologie* 47, 483–490. doi: 10.1007/s13592-015-0413-7

Danka, R. G., Rinderer, T. E., Kuznetsoy, V. N., and Delatte, G. T. (1995). A USDA-ARS project evaluate resistance to *Varroa jacobsoni* by honey bees of far-eastern Russia. *Am. Bee J.* 135, 746–748.

Dietemann, V., Nazzi, F., Martin, S. J., Anderson, D. L., Locke, B., Delaplane, K. S., et al. (2013). Standard methods for *varroa* research. In V Dietemann; JD Ellis; P Neumann (Eds) The Coloss Beebook. Volume I: standard methods for *Apis mellifera* research. *J. Apic. Res.* 52, 1–54. doi: 10.3896/ibra.1.52.1.09

Doublet, V., Labarussias, M., de Miranda, J. R., Moritz, R. F. A., and Pacton, R. J. (2015). Bees under stress: sublethal doses of a neonicotinoid pesticide and pathogens interact to elevate honey bee mortality across the life cycle. *Environ. Microbiol.* 17, 969–983. doi: 10.1111/1462-2920.12426

Eimanifar, A., Brooks, S. A., Bustamante, T., and Ellis, J. D. (2018). Population genomics and morphometric assignment of western honey bees (*Apis mellifera* L.) in the Republic of South Africa. *BMC Genomics* 19:615. doi: 10.1186/s12864-018-4998-x

Eimanifar, A., Pieplow, J. T., Asem, A., and Ellis, J. D. (2020). Genetic diversity and population structure of two subspecies of western honey bees (*Apis mellifera* L.) in the Republic of South Africa as revealed by microsatellite genotyping. *PeerJ* 8:e8280. doi: 10.7717/peerj.8280

Eischen, F. A., Wilson, W. T., Pettis, J. S., Suarez, A., Cardoso-Tamez, D., Maki, D. L., et al. (1990). The Spread of *Acarapis woodi* (*Acari*: Tarsonemidae) in Northeastern Mexico. *J. Kansas Entomol. Soc.* 63, 375–384.

Fahey, R., Rennich, K., Nessa, A., Swan, N., Steinhauer, N., Eversole, H., et al. (2018). *2016 – 2017 National Honey Bee Disease Survey Report. National Honey Bee Disease Survey*. Available online at: https://www.aphis.usda.gov/plant_health/plant_pest_info/honey_bees/downloads/2016-2017-National-Survey-Report.pdf (accessed December 28, 2020).

Fahey, R., Rennich, K., Nessa, A., Swan, N., Steinhauer, N., Eversole, H., et al. (2019). *2017 – 2018 National Honey Bee Disease Survey Report. National Honey Bee Disease Survey. 36pp*. Available online at: https://ushoneybeehealthsurvey.

info/wp-content/uploads/sites/3/2020/04/2017-2018-Summary-Report.pdf (accessed December 28, 2020).

Ferrier, P. M., Rucker, R. R., Thurman, W. N., and Burgett, M. (2018). *Economic Effects and Responses to Changes in Honey Bee Health. USDA Economic Research Service. Economic Research Report Nr. 246. 54 p*. Available online at: https://www.ers.usda.gov/webdocs/publications/88117/err-246.pdf?v=601 (accessed February 15, 2021).

Finger, D., Filho, I. K., Torres, Y. R., and Quinaia, S. P. (2014). Propolis as an indicator of environmental contamination by metals. *Bull. Environ. Contam. Toxicol.* 92, 259–264. doi: 10.1007/s00128-014-1199-4

Galbraith, D. A., Fuller, Z. L., Ray, A. M., Brockmann, A., Frazier, M., Gikungu, M. W., et al. (2018). Investigating the viral ecology of global bee communities with high-throughput metagenomics. *Sci. Rep.* 8:8879. doi: 10.1038/s41598-018-27164-z

Galindo-Cardona, A., Acevedo-Gonzalez, J. P., Rivera-Marchand, B., and Giray, T. (2013). Genetic structure of the gentle Africanized honey bee population (gAHB) in Puerto Rico. *BioMed Central Genet.* 14:65. doi: 10.1186/1471-2156-14-65

Gallai, N., Salles, J. M., Settele, J., and Vaissiere, B. E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecol. Econ.* 68, 810–821. doi: 10.1016/j.ecolecon.2008.06.014

Geslin, B., Gauzens, B., Baude, M., Dajoz, I., Fontaine, C., Henry, M., et al. (2017). Massively introduced managed species and their consequences for plant–pollinator interactions. *Adv. Ecol. Res.* 57, 147–199. doi: 10.1016/bs.aecr.2016.10.007

Giray, T., Guzman-Novoa, E., Aron, C. W., Zelinsky, B., Fahrbach, S. E., and Robinson, G. E. (2000). Genetic variation in worker temporal polyethism and colony defensiveness in the honey bee, *Apis mellifera*. *Behav. Ecol.* 11, 44–55. doi: 10.1093/beheco/11.1.44

Goodrich, B. K., Williams, J. C., and Goodhue, R. E. (2019). The great bee migration: supply analysis of honey bee colony shipments into California for almond pollination Services. *Am. J. Agric. Econ.* 101, 1353–1372. doi: 10.1093/ajae/aaz046

Goretti, E., Pallottini, M., Rossi, R., La Porta, G., Gardi, T., Cenci Goga, B. T., et al. (2020). Heavy metal bioaccumulation in honey bee matrix, an indicator to assess the contamination level in terrestrial environments. *Environ. Pollut.* 256:113388. doi: 10.1016/j.envpol.2019.113388

Goulson, D. (2003). Effects of introduced bees on native ecosystems. *Annu. Rev. Ecol. Evol. Syst.* 43, 1–26. doi: 10.1146/annurev.ecolsys.34.011802.132355

Goulson, D., and Derwent, L. C. (2004). Synergistic interactions between an exotic honey bee and an exotic weed: pollination of *Lantana camara* in Australia. *Weed Res.* 44, 195–202. doi: 10.1111/j.1365-3180.2004.00391.x

Goulson, D., Nicholls, E., Botías, C., and Rotheray, E. L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* 347, 1–16. doi: 10.1126/science.1255957

Granberg, F., Vicente-Rubiano, M., Rubio-Guerri, C., Karlsson, O. E., Kukielka, D., Belák, S., et al. (2013). Metagenomic detection of viral pathogens in spanish honeybees: co-infection by aphid lethal paralysis, israel acute paralysis and lake sinai viruses. *PLoS One* 2013:e57459. doi: 10.1371/journal.pone.0057459

Graystock, P., Blane, E. J., McFrederick, Q. S., Goulson, D., and Hughes, W. O. H. (2016). Do managed bees drive parasite spread and emergence in wild bees? *Int. J. Parasitol.* 5, 64–75. doi: 10.1016/j.ijppaw.2015.10.001

Grozinger, C. M., and Flenniken, M. L. (2019). Bee viruses: ecology, pathogenicity, and impacts. *Annu. Rev. Entomol.* 64, 205–226. doi: 10.1146/annurev-ento-011118-111942

Guimarães, P. R. Jr., Jordano, P., and Thompson, J. N. (2011). Evolution and coevolution in mutualistic networks. *Ecol. Lett.* 14, 877–885. doi: 10.1111/j.1461-0248.2011.01649.x

Guzman-Novoa, E., Morfin, N., De la Mora, A., Macías-Macías, J. O., Tapia-González, J. M., Contreras-Escareño, F., et al. (2020). The process and outcome of the Africanization of honey bees in Mexico: lessons and future directions. *Front. Ecol. Evolut.* 8:608091. doi: 10.3389/fevo.2020.608091

Guzman-Novoa, E., Page, R. E. Jr., and Fondrk, M. K. (1994). Morphometric techniques do not detect intermediate and low levels of Africanization in honey bee (Hymenoptera: Apidae) colonies. *Ann. Entomol. Soc. Am.* 87, 507–515. doi: 10.1093/aesa/87.5.507

Haber, A. I. H., Steinhauer, N. A., and vanEngelsdorp, D. (2019). Use of chemical and nonchemical methods for the control of *Varroa destructor* (Acari: Varroidae) and associated winter colony losses in U.S. beekeeping operations. *J. Econ. Entomol.* 112, 1509–1525. doi: 10.1093/jee/toz088

Hall, G. H. (1992). Suspected African honeybee Colonies in Florida tested for identifying DNA markers. *Fla. Entomol. Soc.* 75, 257–266. doi: 10.2307/3495628

Harbo, J. R., and Harris, J. W. (1999a). Selecting honey bees for resistance to *Varroa jacobsoni*. *Apidologie* 30, 183–196. doi: 10.1051/apido:19990208

Harbo, J. R., and Harris, J. W. (1999b). Heritability in honey bees (Hymenoptera: Apidae) of characteristics associated with resistance to *Varroa jacobsoni* Mesostigmata: Varroidae). *J. Econ. Entomol.* 92, 261–265. doi: 10.1093/jee/92.2.261

Harpur, B. A., Chapman, N. C., Krimus, L., Maciukiewicz, P., Sandhu, V. K., Sood, K., et al. (2015). Assessing patterns of admixture and ancestry in Canadian honey bees. *Insectes Soc* 62, 479–489. doi: 10.1007/s00040-015-0427-1

Hatjina, F., Costa, C., Büchler, R., Uzunov, A., Drazic, M., Filipi, J., et al. (2014). Population dynamics of European honey bee genotypes under different environmental conditions. *J. Apic. Res.* 53, 233–247. doi: 10.3896/ibra.1.53.2.05

Henriques, D., Chávez-Galarza, J., Teixeira, J., Galaschi, S., Ferreira, H., Neves, C. J., et al. (2020). Wing geometric morphometrics of workers and drones and single nucleotide polymorphisms provide similar genetic structure in the Iberian honey bee (*Apis mellifera iberiensis*). *Insects* 11:89. doi: 10.3390/insects11020089

Hopkins, B. K., and Herr, C. (2010). Factors affecting the successful cryopreservation of honey bee (*Apis mellifera*) spermatozoa. *Apidologie.* 41, 548–556. doi: 10.1051/apido/20010006

Hopkins, B. K., Herr, C., and Sheppard, W. S. (2012). Sequential generations of honey bee (*Apis mellifera*) queens produced using cryopreserved semen. *Reprod. Fertil. Dev.* 24, 1079–1083. doi: 10.1071/RD11088

Hung, K.-L. J., Kingston, J. M., Albrecht, M., Holway, D. A., and Kohn, J. R. (2018). The worldwide importance of honey bees as pollinators in natural habitats. *Proc. R. Soc. B* 285:20172140. doi: 10.1098/rspb.2017.2140

Ibrahim, A., and Spivak, M. (2006). The relationship between hygienic behavior and suppression of mite reproduction as honey bee (*Apis mellifera*) mechanisms of resistance to *Varroa destructor*. *Apidologie* 37, 31–40. doi: 10.1051/apido:2005052

Jabr, F. (2013). *The Mind-Boggling Math of Migratory Beekeeping. Scientific American, August 20, 2013*. Available online at: http://www.scientificamerican.com/article/migratory-beekeeping-mind-boggling-math/ (accessed February 15, 2021).

Ji, R., Xie, B., Yang, G., and Li, D. (2003). From introduced species to invasive species—a case study on the Italian bee *Apis mellifera* L. *J. Chin. Ecol.* 5, 70–73.

Jordan, A., Patch, H. M., Grozinger, C. M., and Khanna, V. (2021). Economic Dependence and Vulnerability of United States Agricultural Sector on Insect-Mediated Pollination Service. *Environ. Sci. Technol.* 55, 2243–2253. doi: 10.1021/acs.est.0c04786

Kast, C., Kilchenmann, V., and Droz, B. (2020). Distribution of coumaphos in beeswax after treatment of honeybee colonies with CheckMite® against the parasitical mite *Varroa destructor*. *Apidologie* 51, 112–122. doi: 10.1007/s13592-019-00724-6

Kennedy, C. M., Lonsdorf, E., Neel, M. C., Williams, N. M., Ricketts, T. H., Winfree, R., et al. (2013). A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. *Ecol. Lett.* 16, 584–599. doi: 10.1111/ele.12082

Kerr, W. E. (1967). The history of introduction of African bees to Brazil. *South Afr. Bee J.* 39, 3–5.

Klein, S., Cabirol, A., Devaud, J.-M., Barron, A. B., and Lihoreau, M. (2017). Why bees are so vulnerable to environmental stressors. *Trends Ecol. Evol.* 32, 268–278. doi: 10.1016/j.tree.2016.12.009

Koeniger, G., Koeniger, N., Ellis, J., and Connor, L. (2014). *Mating Biology of Honey Bees (Apis mellifera)*. Kalamazoo, MI: Wicwas Press, 155.

Kovaèiæ, M., Puškadija, Z., Dražiæ, M. M., Uzunov, A., Meixner, M. D., and Büchler, R. (2020). Effects of selection and local adaptation on resilience and economic suitability in *Apis mellifera* carnica. *Apidologie* 51, 1062–1073. doi: 10.1007/s13592-020-00783-0

Lautenbach, S., Seppelt, R., Liebscher, J., and Dormann, C. F. (2012). Spatial and temporal trends of global pollination benefit. *PLoS One* 7:e35954. doi: 10.1371/journal.pone.0035954

Losey, J. E., and Vaughan, M. (2006). The economic value of ecological services provided by insects. *BioScience* 56, 311–323. doi: 10.1641/0006-3568(2006)56[311:tevoes]2.0.co;2

Mailander, D., and Grant, Z. (2019). *When Honey Bees Hit the Road: The Role of Federal, State, and Local Laws in Regulating Honey Bee Transportation. A White Paper of the University of Oregon School of Law Environmental and Natural Resources Law Center Food Resiliency Projec. University of Oregon. 55 p*. Available online at: https://law.uoregon.edu/sites/law1.uoregon.edu/files/when_honey_bees_hit_the_road.pdf (accessed April 26, 2022).

Mallinger, R. E., Gaines-Day, H. R., and Gratton, C. (2017). Do managed bees have negative effects on wild bees? A systematic review of the literature. *PLoS One* 12:e0189268. doi: 10.1371/journal.pone.0189268

Matthews, W. A., Baratashvili, S., and Sumner, D. A. (2020). *University of California, Agricultural Issues Center. Contributions of the California Almond Industry to the California Economy. Report for the Almond Board of California*. Available online at: https://aic.ucdavis.edu/wp-content/uploads/2020/08/Economic-Impact-Study-Manuscript-Final.pdf (accessed April 26, 2022).

McKinney, M. L., and La Sorte, F. A. (2007). Invasiveness and homogenization: synergism of wide dispersal and high local abundance. *Glob. Ecol. Biogeogr.* 16, 394–400. doi: 10.1111/j.1466-8238.2007.000296.x

Meixner, M. D., Pinto, M. A., Bouga, M., Kryger, P., Ivanova, E., and Fuchs, S. (2013). Standard methods for characterizing subspecies and ecotypes of *Apis mellifera*. *J. Apic. Res.* 52, 1–27. doi: 10.3896/ibra.1.52.4.05

Meusel, M. S., and Moritz, R. F. A. (1993). Transfer of paternal mitochondrial DNA during fertilization of honeybee (*Apis mellifera* L.) eggs. *Curr. Genet.* 24, 539–543. doi: 10.1007/BF00351719

Millán-Leiva, A., Marín, Ó, Christmon, K., vanEngelsdorp, D., and González-Cabrera, J. (2021). Mutations associated with pyrethroid resistance in *Varroa* mite, a parasite of honey bees, are widespread across the United States. *Pest. Manag. Sci.* 77, 3241–3249. doi: 10.1002/ps.6366

Momeni, J., Parejo, M., Nielsen, R. O., Langa, J., Montes, I., Papoutsis, L., et al. (2021). Authoritative subspecies diagnosis tool for European honey bees based on ancestry informative SNPs. *BMC Genomics* 22:101. doi: 10.1186/s12864-021-07379-7

Mondet, F., Beaurepaire, A., McAfee, A., Locke, B., Alaux, C., Blanchard, S., et al. (2020). Honey bee survival mechanisms against the parasite *Varroa destructor*: a systematic review of phenotypic and genomic research efforts. *Int. J. Parasitol.* 50, 433–447. doi: 10.1016/j.ijpara.2020.03.005

Moritz, R. F. A., Hartel, S., and Neumann, P. (2005). Global invasions of the western honeybee (*Apis mellifera*) and the consequences for biodiversity. *Ecoscience* 12, 289–301. doi: 10.2980/i1195-6860-12-3-289.1

Mullin, C. A., Frazier, M., Frazier, J. L., Ashcraft, S., Simonds, R., vanEngelsdorp, D., et al. (2010). High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. *PLoS One* 5:e9754. doi: 10.1371/journal.pone.0009754

Munoz, I., Henriques, D., Johnston, J. S., Chavez-Galarza, J., Kryger, P., and Pinto, M. A. (2015). Reduced SNP panels for genetic identification and introgression analysis in the dark honey bee (*Apis mellifera mellifera*). *PLoS One* 10:e0124365. doi: 10.1371/journal.pone.0124365

NASS (2020). *National Agricultural Statistics Service. United States Department of Agriculture. National Statistics for Almonds. 2020 California Almond Objective Measurement Report*. Available online at: https://www.nass.usda.gov/Statistics_by_State/California/Publications/Specialty_and_Other_Releases/Almond/Objective-Measurement/202007almom.pdf (accessed February 16, 2021].

NASS (2021). *National Agricultural Statistics Service. United States Department of Agriculture. United States Honey Production*. Available online at: https://downloads.usda.library.cornell.edu/usda-esmis/files/hd76s004z/7h14bh90x/w9505v43v/hony0321.pdf (accessed April 26, 2022).

Nature. (1912). The Isle of wight bee disease. *Nature* 89:410. doi: 10.1038/089410a0

Nawrocka, A., Kandemir, I., Fuchs, S., and Tofilski, A. (2018). Computer software for identification of honey bee subspecies and evolutionary lineages. *Apidologie* 49, 172–184.

Neumann, P. (2006). Infestation levels of *Apis mellifera scutellata* swarms by socially parasitic Cape honeybee workers (*Apis mellifera capensis*). *Apidologie* 37, 462–470. doi: 10.1051/apido:2006012

Neumann, P., and Moritz, R. F. A. (2002). The cape honeybee phenomenon: the sympatric evolution of a social parasite in real time? *Beahv. Ecol. Sociobiol.* 52, 271–281. doi: 10.1007/s00265-002-0518-7

Norfolk, O., Gilbert, F., and Eichhorn, M. P. (2018). Alien honey bees increase pollination risks for range-restricted plants. *Divers. Distrib.* 24, 705–713. doi: 10.1111/ddi.12715

Owen, R. (2017). Role of human action in the spread of honey bee (Hymenoptera: Apidae) pathogens. *J. Econ. Entomol.* 110, 797–801. doi: 10.1093/jee/tox075

Paini, D. R., and Roberts, J. D. (2005). Commercial honey bees (*Apis mellifera*) reduce the fecundity of an Australian native bee (*Hylaeus alcyoneus*). *Biol. Conserv.* 123, 103–112. doi: 10.1016/j.biocon.2004.11.001

Perez, A., and Plattner, K. (2014). *Fruit and Tree Outlook. USDA Economic Research Service. Fruit and Tree Nuts Outlook Special Article/FTS-357SA/September 26, 2014*. Available online at: https://www.ers.usda.gov/webdocs/outlooks/37059/49130_fts-357.pdf?v=6225.6 (accessed September 26, 2014).

Pernal, S. F. (2014). "National regulations for beekeeping in north america (Canada and the United States of America)," in *Bee Health and Veterinarians*, ed. W. Ritter (Paris: OIE World Organization for Animal Health), 275–280.

Phillips, E. F. (1920). Control of American foulbrood. *Farmers Bull.* 1084, 1–16.

Pilati, L., and Prestamburgo, M. (2016). Sequential relationship between profitability and sustainability: the case of migratory beekeeping. *Sustainability* 8:94. doi: 10.3390/su8010094

Pinto, M. A., Henriques, D., Chaves-Galarza, J., Kryger, P., Garnery, L., Van der Zee, M., et al. (2014). Genetic integrity of the Dark European honey bee (*Apis mellifera mellifera*) from protected populations: a genome-wide assessment using SNPs and mtDNA sequence data. *J. Apic. Res.* 53, 269–278. doi: 10.3896/ibra.1.53.2.08

Ramadan, M. M., Reimer, N. J., Oishi, D. E., Young, C. L., and Heu, R. A. (2019). *Varroa mite Varroa destructor* Anderson & Trueman (Acari: Varroidae). New York, NY: Springer.

Ray, A. M., Lopez, D. L., Iturralde Martinez, F. J., Galbraith, D. A., Rose, R., Van Engelsdorp, D., et al. (2020). Distribution of recently identified bee-infecting viruses in managed honey bee (*Apis mellifera*) populations in the USA. *Apidologie* 51, 736–745. doi: 10.1007/s13592-020-00757-2

Rennie, J. (1921). Isle of wight disease in hive bees – acarine disease: the organism associated with the disease *Tarsonemus woodi*, n. sp. *Trans. R. Soc. Edinburgh* 52, 768–779. doi: 10.1017/s0080456800016008

Ribani, A., Utzeri, V. J., Taurisano, V., and Fontanesi, L. (2020). Honey as a source of environmental DNA for the detection and monitoring of honey bee pathogens and parasites. *Vet. Sci.* 7:113. doi: 10.3390/vetsci7030113

Rinderer, T. E., and Coy, S. E. (2020). *Russian Honey Bees*. Salmon Bayou Press, 230.

Rinderer, T. E., de Guzman, L. I., Harris, J., Kuznetsov, V., Delatte, G. T., Stelzer, J. A., et al. (2000). The release of ARS Russian honey bees. *Am. Bee J.* 140, 305–307.

Rinderer, T. E., Delatte, G. T., De Guzman, L. I., Williams, J., Stelzer, J. A., and Kuznetsov, V. N. (1999). Evaluations of the *Varroa*-resistance of honey bees imported from Far-Eastern Russia. *Am. Bee J.* 139, 287–290.

Rinderer, T. E., Guzman, L. I., Delatte, G. T., Stelzer, J. A., Williams, J. L., Beaman, L. D., et al. (2001a). Multi-state field trials of ARS Russian honey bees 1. Response to *Varroa destructor* 1999, 2000. *Am. Bee J.* 141, 658–661.

Rinderer, T. E., Guzman, L. I., Delatte, G. T., Stelzer, J. A., Williams, J. L., Beaman, L. D., et al. (2001b). Multi-state field trials of ARS Russian honey bees 2. Honey production 1999, 2000. *Am. Bee J.* 141, 726–729.

Rinderer, T. E., Kuznetsoy, V., Danka, R. G., and Delatte, G. T. (1997). An importation of potentially *Varroa*-resistant honey bees from Far-Eastern Russia. *Am. Bee J.* 137, 787–789.

Rinkevich, F. D. (2020). Detection of amitraz resistance and reduced treatment efficacy in the *Varroa* Mite, *Varroa destructor*, within commercial beekeeping operations. *PLoS One* 15:e0227264. doi: 10.1371/journal.pone.0227264

Rivera-Marchand, B., Oskay, D., and Giray, T. (2012). Gentle Africanized bees on an oceanic island. *Evol. Appl.* 5, 746–756. doi: 10.1111/j.1752-4571.2012.00252.x

Roubik, D. (2009). Ecological impact on native bees by the invasive Africanized honey bee. *Acta Biol. Colombiana* 14, 114–124.

Roubik, D., and Villanueva-Gutierrez, R. (2009). Invasive Africanized honey bee impact on native solitary bees: a pollen resource and trap nest analysis: responses to invasive honey bees. *Biol. J. Linnean Soc.* 98, 152–160. doi: 10.1111/j.1095-8312.2009.01275.x

Rucker, R. R., Thurman, W. N., and Burgett, M. (2012). Honey bee pollination markets and the internalization of reciprocal benefits. *Am. J. Agric. Econ.* 94, 956–977. doi: 10.1093/ajae/aas031

Rules and Regulations (2014). United States department of agriculture. Animal and plant health inspection services. 7 CFR parts 319 and 322. Bees and related articles. *Federal Register* 69, 61735–61755.

Runckel, C., Flenniken, M. L., Engel, J. C., Ruby, J. G., Ganem, D., Andino, R., et al. (2011). Temporal analysis of the honey bee microbiome reveals four novel viruses and seasonal prevalence of known viruses, *Nosema, and Crithidia*. *PLoS One* 6:e20656. doi: 10.1371/journal.pone.0020656

Rusert, L. M., Pettis, J. S., and Tarpy, D. R. (2021). Introduction of *Varroa destructor* has not altered honey bee queen mating success in the Hawaiian archipelago. *Sci. Rep.* 11:1366. doi: 10.1038/s41598-020-80525-5

Russo, L. (2016). Positive and negative impacts of non-native bee species around the world. *Insects* 7:69. doi: 10.3390/insects7040069

Sadd, B. M., Kleinlogel, Y., Schmid-Hempel, R., and Schmid-Hempel, P. (2005). Trans-generational immune priming in a social insect. *Biol. Lett.* 1, 386–388. doi: 10.1098/rsbl.2005.0369

Schiff, N. M., and Sheppard, W. S. (1995). Genetic Analysis of commercial honey bees (Hymenoptera: Apidae) from the southeastern United States. *J. Econ. Entomol.* 88, 1216–1220. doi: 10.1093/jee/88.5.1216

Schneider, S. S., DeGrandi-Hoffman, G., and Smith, D. R. (2004). The African Honey bee. Factors contributing to a successful biological invasion. *Ann. Rev. Entomol.* 49, 351–376. doi: 10.1146/annurev.ento.49.061802.123359

Shahbandeh, M. (2021). *Honey Market Worldwide And In The U.S. – Statistics & Facts. Statista*. Available online at: https://www.statista.com/topics/5090/honey-market-worldwide/#dossierKeyfigures (accessed October 1, 2021).

Sheppard, W. S. (1989a). A history of the introduction of honey bee races into the United Sates: Part I. *Am. Bee J.* 129, 617–619.

Sheppard, W. S. (1989b). A history of the introduction of honey bee races into the United Sates: Part II. *Am. Bee J.* 129, 664–667.

Sheppard, W. S. (2012). Honey bee genetic diversity and breeding: towards the reintroduction of european germplasm. *Bee Health* 140, 24–27.

Shimanuki, H., and Knox, D. A. (2000). *Diagnosis of Honey Bee Diseases. Agriculture Handbook No. AH690*. Beltsville, MD: United States Department of Agriculture.

Simone-Finstrom, M., Li-Byarlay, H., Huang, M. H., Strand, M. K., Rueppell, O., and Tarpy, D. R. (2016). Migratory management and environmental conditions affect lifespan and oxidative stress in honey bees. *Sci. Rep.* 6:32023. doi: 10.1038/srep32023

Smith, D. R. (1991). African bees in the americas: insights from biogeography and genetics. *Trends Ecol. Evol.* 6, 17–21. doi: 10.1016/0169-5347(91)90142-K

Spivak, M., and Reuter, G. S. (2001). Resistance to American foulbrood disease by honey bee colonies *Apis mellifera* bred for hygienic behavior. *Apidologie* 32, 555–565. doi: 10.1051/apido:2001103

Spivak, M., and Reuter, G. S. (2016). *Honey bee Diseases and Pests: A companion to Beekeeping in Northern Climates*. St Paul, MN: University of Minesota Extension, 42.

Steinhauer, N., vanEngelsdorp, D., and Saegerman, C. (2020). Prioritizing changes in management practices associated with reduced winter honey bee colony losses for US beekeepers. *Sci. Total Environ.* 753:141629. doi: 10.1016/j.scitotenv.2020.141629

Strauss, U., Dietemann, V., Human, H., Crewe, R. M., and Pirk, C. W. W. (2015). Resistance rather than tolerance explains survival of savannah honeybees (*Apis mellifera scutellata*) to infestation by the parasitic mite *Varroa destructor*. *Parasitology* 143, 374–387. doi: 10.1017/S0031182015001754

Sumner, D. A., Matthews, W. A., Medellín-Azuara, J., and Bradley, A. (2014). *The Economic Impacts of the California Almond Industry. The Almond Board of California*. Davis, CA: University, California.

Surface, H. A. (1916). Bee diseases in Pennsylvania. *Zool. Bull.* 6, 1–23.

Thomson, D. (2004). Competitive interactions between the invasive European honey bee and native bumble bees. *Ecology* 85, 458–470. doi: 10.1890/02-0626

Thomson, D. M. (2016). Local bumble bee decline linked to recovery of honey bees, drought effects on floral resources. *Ecol. Lett.* 19, 1247–1255. doi: 10.1111/ele.12659

Torné-Noguera, A., Rodrigo, A., Osorio, S., and Bosch, J. (2016). Collateral effects of beekeeping: Impacts on pollen-nectar resources and wild bee communities. *Basic Appl. Ecol.* 17, 199–209. doi: 10.1016/j.baae.2015.11.004

Traniello, J. F. A., Rosengaus, R. B., and Savoie, K. (2002). The development of immunity in a social insect: evidence for the group facilitation of disease resistance. *Proc. Natl. Acad. Sci. U.S.A.* 99, 6838–6842. doi: 10.1073/pnas.102176599

Traynor, K. S., Rennich, K., Forsgren, E., Rose, R., Pettis, J., Kunkel, G., et al. (2016). Multiyear survey targeting disease incidence in US honey bees. *Apidologie* 47, 325–347. doi: 10.1007/s13592-016-0431-0

Traynor, K. S., Tosi, S., Rennich, K., Steinhauer, N., Forsgren, E., Rose, R., et al. (2021). Pesticides in honey bee colonies: establishing a baseline for real world exposure over seven years in the USA. *Environ. Pollut.* 279:116566. doi: 10.1016/j.envpol.2021.116566

USDA (2020). *Honey Bee Colonies*. Washington, DC: USDA.

Uzunov, A., Costa, C., Panasiuk, B., Meixner, M., Kryger, P., Hatjina, F., et al. (2014). Swarming, defensive and hygienic behaviour in honey bee colonies of different genetic origin in a Pan-European experiment. *J. Apic. Res.* 53, 248–260. doi: 10.3896/ibra.1.53.2.06

Valido, A., Rodríguez-Rodríguez, M. C., and Jordano, P. (2019). Honeybees disrupt the structure and functionality of plant-pollinator networks. *Sci. Rep.* 9:4711. doi: 10.1038/s41598-019-41271-5

Van der Steen, J., de Kraker, J., and Grotenhuis, T. (2011). Spatial and temporal variation of metal concentrations in adult honeybees (*Apis mellifera* L.). *Environ. Monit. Assessment* 184, 4119–4126. doi: 10.1007/s10661-011-2248-7

vanEngelsdorp, D., and Meixner, M. D. (2010). A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. *J. Invertebr. Pathol.* 103, S80–S95. doi: 10.1016/j.jip.2009.06.011

vanEngelsdorp, D., Tarpy, D. R., Lengerich, E. J., and Pettis, J. S. (2012). Idiopathic brood disease syndrome and queen events as precursors of colony mortality in migratory beekeeping operations in the eastern United States. *Prev. Vet. Med.* 108, 225–233. doi: 10.1016/j.prevetmed.2012.08.004

Visscher, P. K., Vetter, R. S., and Baptista, F. C. (1997). Africanized bees, 1990-1995: Initial rapid expansion has slowed in the U.S. *Calif. Agric* 51, 22–25. doi: 10.3733/ca.v051n01p22

Voorhies, E. C., Todd, Frank, E., and Galbraith, J. K. (1933). Economic aspects of the bee industry. *Univer.Calif. Coll. Agric. Bull.* 555, 1–117.

VPPC (2016). *Vermont's pollination Protection committee. Report to the Vermont Legislature as required by Act 83 of 2016 Session. 67 p*. Available online at: https://agriculture.vermont.gov/sites/agriculture/files/documents/Pollinator%20Protection%20Report-FINAL.pdf (accessed February 16 2021).

Wehling, W. F., and Flanders, R. V. (2005). *The Risks of International Trade in Bees: Laws And Regulations on The Import, Movement and Release Of Bees in The United States. Proceedings of The Bee Research and Virus in Europe (BRAVE), Sophia-Antipolis, France, 24-26 April 2005, page 301-309*. Available online at: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.115.4234&rep=rep1&type=pdf#page=301 (accessed April 26, 2022).

Whitfield, C. W., Behura, S. K., Berlocher, S. H., Clark, A. G., Johnston, J. S., Sheppard, W. S., et al. (2006). Thrice out of Africa: ancient and recent expansions of the honey bee, *Apis mellifera*. *Science* 314, 642–645. doi: 10.1126/science.1132772

Williams, I. H. (1994). The dependences of crop production within the European Union on pollination by honey bees. *Agric. Zool. Rev.* 6, 229–257.

Winter, K., Adams, L., Thorp, R., Inouye, D., Day, L., Ascher, J., et al. (2006). *Importation of Non-Native Bumble Bees into North America: Potential Consequences of Using Bombus terrestris and Other Non-Native Bumble Bees for Greenhouse Crop Pollination in Canada, Mexico, and the United States. A White Paper of the North American Pollinator Protection Campaign (NAPPC)*. Available online at: https://www.pollinator.org/pollinator.org/assets/generalFiles/BEEIMPORTATION_AUG2006.pdf (accessed April 26, 2022).

Zhu, X., Zhou, S., and Huang, Z. Y. (2014). Transportation and pollination service increase abundance and prevalence of *Nosema ceranae* in honey bees (*Apis mellifera*). *J. Apic. Res.* 53, 469–471. doi: 10.3896/ibra.1.53.4.06

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