SOCIAL INVERTEBRATES AS MODELS FOR NON-KIN COOPERATION

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SOCIAL INVERTEBRATES AS MODELS FOR NON-KIN COOPERATION

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Long-Term Colony Dynamics and Fitness in a Colonial Tent-Web Spider *Cyrtophora citricola*

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Social animals are expected to experience a positive effect of conspecific number or density on fitness (an Allee effect) because of the benefits of group living. However, social animals also often disperse to live either solitarily or in small groups, so to understand why social animals leave their groups it is necessary to understand how group size affects both average fitness and the expected fitness outcomes of individuals. We examined the relationships between group size and fitness in the colonial spider *Cyrtophora citricola* using long-term observations of colony demographics. We censused colonies, recording the number of juveniles, large females, and egg sacs, approximately every 2 months for 2 years. We also recorded the substrates supporting colony webs, including plant species and size, and the azimuth the colony occupied on the plant. Colonies in all regions showed cyclical patterns of growth and decline; however, regions were not synchronized, and seasonal effects differed between years. Colonies with fewer individuals at the initial observation were less likely to survive over the course of observations, and extinction rates were also influenced by an interaction between region and plant substrate. Small colonies were more likely to be extinct by the next census, but if they survived, they were more likely to have high growth rates compared to larger colonies. Despite the potential for high growth rates, high extinction rates depressed the average fitness of small colonies so that population growth rates peaked at intermediate colony sizes. Variance in egg sac production also peaked at intermediate colony sizes, suggesting that competitive interactions may increase the uneven distribution of resources in larger groups. Even if average fitness is high, if spiders can anticipate poor outcomes in large colonies, they may disperse to live solitarily or in smaller, less competitive groups.

Keywords: Allee effect, colonization, extinction, reproductive skew, social, spider, demographics, population dynamics

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INTRODUCTION

Societies only evolve when the benefits of living in groups outweigh the substantial costs, such as increased competition and parasite transmission (Alexander, 1974; Krause and Ruxton, 2002). The ubiquity of social organization, from slime molds to humans (Samuelson, 2005; Bonner, 2009), indicates that these benefits, both active (e.g., cooperative prey capture) and passive (e.g., predator dilution), readily accrue in a variety of ecological contexts. However, social organisms face a problem. No environment will remain suitable forever, so all organisms must disperse to colonize new habitats (Méndez et al., 2014). For social organisms, this may necessitate individuals separating from groups and the advantages they provide. Some societies have solved this problem by colony fission and group dispersal (e.g., honey bees; Winston, 1987), but for many others, particularly those with limited control over dispersal (e.g., ballooning spiders), dispersal is a solitary endeavor (Tschinkel and Howard, 1983; Schneider et al., 2001; Chapple, 2003; Schoepf and Schradin, 2012). To understand how these societies grow, decline, and colonize new sites, where they may survive at necessarily low populations, we must understand how group size influences individual fitness, fitness variation, and the probability of group extinction.

An Allee effect is a positive effect of conspecific number or density on fitness or a component of fitness (Stephens et al., 1999). Although expected in any obligate sexual organism (due to a need to find mates), Allee effects are thought to be particularly strong in social species (Angulo et al., 2018), where solitary individuals or small groups may fail to reap the benefits of group living. But if leaving the group entails fitness costs, why then do some individuals of social species disperse to live solitarily or at sub-optimal group sizes? One potential reason is that reproduction within groups is often distributed unevenly (i.e., there is high reproductive skew). Reproductive skew theory predicts when subordinates (individuals expected to have relatively low reproduction in the group) should stay in the group or attempt solitary breeding (Reeve and Shen, 2013). The higher the constraints on solitary breeding, the more likely a subordinate is to stay in the group. But even if the average fitness of the group is high, if the expected payoff for the subordinate is below what could be achieved alone, the subordinate should leave (Reeve and Shen, 2006). In social species that frequently disperse to live alone or colonize new habitats with low populations, we expect two patterns: (1) Allee effects will increase average individual fitness with increasing group size (at least up to a point), and (2) reproductive skew will be higher in larger groups, as antagonistic interactions between dominant and subordinate individuals increase with increasing density. If groups provide both higher average fitness and egalitarian fitness allocation, there would be little reason to disperse and lose fitness advantages. This is particularly true in non-kin societies with reduced risk of inbreeding and kin competition, both of which can promote dispersal (Bowler and Benton, 2005).

We tested these hypotheses in a colonial tent-web spider, *Cyrtophora citricola*, using long-term monitoring of natural colony demographics. Like other colonial spider species, *C. citricola* spiders preferentially live in aggregations of webs built, maintained, and defended by individual spiders (Uetz and Hieber, 1997; Mestre and Lubin, 2011; Yip et al., 2017). There is little cooperative prey capture or brood care, but conspecifics are tolerated on support threads that connect neighboring webs (Uetz and Hieber, 1997). Despite little active cooperation, spiders may derive several benefits from grouping. In *C. citricola*, prey capture efficiency increases with colony size (Rypstra, 1979, but see Leborgne et al., 1998). While not conclusively demonstrated in *C. citricola*, other colonial spider species benefit from early warning of predators (Uetz and Hieber, 1997), protection from predators via the "selfish herd" (Rayor and Uetz, 1993), and reduced prey capture variance (Caraco et al., 1995; Uetz, 1996). Although some studies have examined the relationship between colony size and fitness in colonial spider species (e.g., Uetz and Hieber, 1997) and others have observed colonies over the course of several months (e.g., Rayor and Uetz, 1993; Grinsted et al., 2019), no study has documented long-term colony growth and decline, so it remains unclear how proposed costs and benefits of group living play out for the dynamics of group size.

Cyrtophora citricola colonies can comprise up to several thousand individuals, but individuals are also found singly (Mestre and Lubin, 2011). Colonies in a semi-natural environment grew by a combination of natal philopatry and immigration (Yip et al., 2019), and genetic analyses indicated considerable variation in genetic structure of natural colonies (Johannesen et al., 2012). In nature, spiders probably encounter a combination of kin and non-kin, and juvenile dispersal—particularly within a limited range—is common (Johannesen et al., 2012; Yip et al., 2019). Thus, *C. citricola* spiders can choose to stay in established colonies or disperse to live in smaller groups and potentially colonize new areas. Previous work on this species found that dispersal behavior is negatively density dependent, suggesting that Allee effects influence dispersal decisions (Ventura et al., 2017). In addition to local dispersal, *C. citricola* has successfully colonized multiple locations in North and South America and Caribbean islands from its native Asian, African, and circum-Mediterranean range (Chuang and Leppanen, 2018). Despite potentially strong Allee effects, its history of invasion success suggests that *C. citricola* spiders can have high fitness at small population sizes.

We tested whether high reproductive skew in larger colonies might explain dispersal to smaller groups or solitary living by censusing natural colonies repeatedly over two years. If Allee effects are driving colony dynamics and spider fitness, we predicted that colony growth and average reproduction would peak at large or intermediate colony sizes. We also predicted that variation in reproduction should increase with colony size, as increased interaction between spiders of differing competitive ability results in greater reproductive skew. If individuals can anticipate poor outcomes, high reproductive skew in colonies with high average fitness may explain dispersal to live solitarily or in smaller, less competitive groups. To understand how *C. citricola* invades new environments, we also measured Allee effects at the population level. Because there is little interaction between sessile colonies at a site, we predicted that Allee effects at the group level may not carry over to the population, i.e., small populations may grow as well as larger populations. We also investigated factors that influence the rate of colony extinction and recolonization. Larger colonies may be resistant to extinction, and recolonization rates provide a measure of dispersal to suitable substrates. To take into account local conditions, we assessed how seasonality and substrate features may affect group size and colony dynamics.

MATERIALS AND METHODS

Cyrtophora citricola is a colonial araneid that builds a horizontal orb web, with support threads above and below that may connect to the substrate or other neighboring webs. Unlike most other araneids, the web of *C. citricola* lacks sticky glue droplets. Under laboratory conditions, females mature 150–200 days after hatching, while males mature after only 60–80 days (Yip and Lubin, 2016). After maturing, females can live another 300 days in the laboratory. The much smaller males (2–2.5 mm body length, compared to 8 mm for females) are usually eaten by females after their first copulation (Yip et al., 2016), but if they do not mate, they can live over 200 days after maturity (Yip and Lubin, 2016). Juveniles are tolerated in support threads of their mother's web, but mothers do not provision offspring or discriminate against unrelated juveniles (Blanke, 1972; Yip et al., 2019). To our knowledge, data reported here are the first to describe the seasonal life cycle of *C. citricola*.

We selected seven sites across southern Israel, along an east to west gradient (**Figure 1A** and **Table 1**). We divided sites into three regions: West Negev (Shuva, Bessor and the central Negev sites of Beer Sheva and Retamim), East Negev (Nahal Gov and Mishor Yamin), and the Arava Valley. At each site, we flagged *C. citricola* colonies and solitary individuals for repeated observations (total colony $n = 126$). Censuses began in either November or December, 2012 and were repeated approximately every two months for two years (see **Supplementary Table 1** for all census dates).

At each census, we counted the number of spiders in every flagged colony and added new colonies if they appeared within the census area. Note that "colony size" throughout refers to the number of spiders in the colony, whereas "web volume" indicates the physical dimensions of the colony. For simplicity, we also use "colony" to refer to both groups of spiders and singletons (a group size of one). We counted adult females guarding egg sacs, large females that were either subadults (last pre-adult instar) or adults that did not have egg sacs, and juveniles or males. Because males are so small, they are easily mistaken for juveniles from a distance, so males and juveniles could not be reliably distinguished (Yip and Lubin, 2016). However, based on colonies we could observe closely, the vast majority of small spiders were juveniles. We recorded the number of egg sacs guarded by females. We also recorded unguarded egg sacs, but these appeared old and empty and were not included in our analyses. For small groups, we counted every individual. For large colonies (estimated population sizes >150) or for portions of colonies that we could not get close enough to census, we censused accessible portions of the colony using a 0.125 m^3 frame (0.5 m × 0.5 m × 0.5 m) and counted all individuals in the framed volume. We sampled areas with similar web density to uncensused portions of the colony at 3–6 locations depending on the size of the colony, with frames spaced evenly along the colony. We then used tape measures and extendable poles (for elevated areas) to estimate the rectangular volume of uncensused web. We estimated the total number of spiders in the colony by multiplying the total volume of web by the spider density in the sampled areas.

In addition to spiders and their egg sacs, for the first census of every colony we recorded the colony substrate (usually plant genus), the size of the substrate (usually plant crown area), and the azimuth, or compass bearing of the colony's position around the center of the substrate (**Figure 1B**). We categorized substrates as *Vachellia* (*V. tortilis, V. raddiana, or V gerrardii*), "cactus," of which Pitaya (*Stenocereus* spp.) was most common, clementine trees, or "shrub" (most other plant genera).

Analyses

All analyses were conducted using R version 4.0.3. We compared colony azimuth among sites using circular ANOVA followed by pairwise comparisons and tested whether orientations differed from random using Rao's spacing test of uniformity (using package "circular").

To analyze the effect of season on colony demographics, we categorized seasons as winter (December–February), spring (March–May), summer (June–August), and fall (September–November). We standardized colony size for each colony by subtracting the mean colony size from each observation and dividing by the standard deviation, so that a value of zero indicated the long-term average size of a particular colony. Positive values indicated the colony was larger than its long-term average, and negative values indicated that it was smaller. Standardization prevented changes in the demographics of large colonies from swamping the effects of smaller colonies. We then examined how standardized numbers of individuals varied by season, year, region, and age class of spider (females with eggs, large females, and juveniles plus males) using linear mixed models with colony ID as a random effect to account for repeated measures over time ("lmer" function in the package "lme4"). We took *P*-values from likelihood ratio tests by comparing the full model to the model without the fixed effect of interest.

We used right-censored data and Cox proportional hazard models to analyze colony extinction and recolonization ("coxph" function in the "survival" package). We tested whether risk of extinction correlated with colony size at the first observation, substrate size and type, region, and the number of degrees off the preferred azimuth for the site. Note that we divided our sites *a priori* into three regions (**Table 1**); where region had significant effects, we also looked at the effect of site to see if particular sites were driving these patterns. We used similar models to examine time to recolonization for colonies that had gone extinct, where the time to the event was measured from disappearance of spiders to reappearance at a flagged colony. We tested whether chances of recolonization correlated to substrate size and type, previous colony size (as a proxy for web remnants), and region. For continuous variables and categorical variables with only two levels, we used z-tests to calculate *P*-values. For categorical variables with more than two levels, we used likelihood ratio tests by comparing the full model to the model without the effect of interest.

We examined how colony size correlated with colony growth in two ways. We examined the average number of egg sacs

TABLE 1 | Census locations, total observation times, and number of colonies observed by site and region.

Site			Coordinates	First census date	Last census date	Number of colonies	Number of censuses	Total time span (days)
Arava	Arava	Site 1	N30d, 43.117 m; E35d, 116.899 m	19-Nov-12	21-Aug-14	6	10	640
		Site 2	N30d, 42.258 m; E35d, 16.287 m	19-Nov-12	21-Aug-14	12	10	640
		Site 3	N30d, 40.645 m; E35d, 14.307 m	19-Nov-12	27-Aug-14	16	10	646
		Site 4	N30d, 37.937 m; E35d, 13.029 m	19-Nov-12	27-Aug-14	15	10	646
		Site 5	N30d, 38.266 m, E 35d, 13.826 m	31-Mar-14	27-Aug-14	1	3	149
W. Negev	Beer Sheva Zoo		N31d, 15.568 m; E34d, 44.631 m	10-Dec-12	9-Sep-14	6	10	638
	Bessor Reserve		N31d, 13.552 m; E34d, 30.605 m	6-Dec-12	25-Nov-14	11	10	719
	Retamim		N31d, 3.313 m; E34d, 42.643 m	6-Dec-12	9-Sep-14	32	8	642
	Shuva		N31d, 27.703 m; E34d, 31.92 m	29-Jan-13	25-Nov-13	7	6	300
E. Negev	Mishor Yamin		N30d, 56.160 m; E35d, 7.179 m	3-Dec-12	21-Aug-14	10	10	626
	Nahal Gov		N30d, 54.944 m; E35d, 8.047 m	3-Dec-12	21-Aug-14	10	10	626

Number of colonies includes colonies added throughout the study.

per female (both females guarding egg sacs and large females without egg sacs) by the number of females in the colony using linear mixed models. We included colony ID as a random effect (both intercept and slope with respect to colony size) to account for repeated observations over time, and we included season, year, and their interaction as covariates to account for seasonality (see section "Results"). The average number of sacs per female and total number of females were natural log transformed to normalize residuals. We also examined the percent change in total number of spiders from one census to the next. As with the egg sac model, we included colony ID as a random effect, included year, season, and their interaction as covariates, and natural log transformed data to normalize residuals. We used a similar model to test for population-level Allee effects, but instead of examining changes in colony size, we analyzed how the total population of a site correlated with the change in site population size between censuses.

Finally, to examine how colony size correlated with variation in reproduction, we calculated the coefficient of variation (CV) of egg sac production among females within each colony for each census date. Singletons were sufficiently rare that many dates had only one singleton with eggs, so to measure the variation in reproduction for singletons, we calculated the CV for all singletons at each site across dates. We then used linear mixed models to test for correlations between CV and either the number of large females in the colony (females with and without eggs) and only females that were guarding egg sacs. Colony ID was a random effect with both intercept and slope with respect to the number of females in the colony, and year and season were included as covariates.

RESULTS

Substrate Size, Type, and Azimuth

We found colonies on a variety of trees, shrubs and cacti. In undisturbed sites, *C. citricola* colonies occupied *Vachellia* spp. trees and shrubs of *Hammada* spp. Less frequently, colonies occupied shrubs of the genera *Salsola, Prosopis*, *Anabisis*, *Ochradenus*, *Retama*, *Haloxylan*, *Atriplex*, *Atractylis*, and *Zygophyllum* (in order of decreasing frequency; see **Supplementary Table 2**). In agricultural and disturbed areas, colonies were found on planted Pitaya (*Stenocereus* spp.) and other cacti, *Vachellia gerrardii*, clementine trees, and *Juniperus* sp. bushes. While colonies occasionally spanned more than one plant, these plants were always very close together. Colony webs did not span wide spaces between substrates, resulting in a correlation between substrate size and colony size (linear model: $df = 123$, $t = 6.8$, $P < 0.0001$, $R^2 = 0.27$; **Figure 2**). Colony sizes varied from singletons to an estimated 28,400 spiders occupying a *Vachellia tortilis* tree with a crown area of 222 m^2 and an estimated total web volume of 205 m^3. The overall average azimuth of colonies was southeast (119° clockwise off north), and this preference was significant (Rao's spacing test: $P < 0.001$; **Figure 1A**). Average azimuth differed among sites (circular ANOVA $F_{5,69} = 4.5$, $P = 0.001$), with pairwise comparisons indicating that Retamim and Shuva had colonies oriented more toward the east compared to other sites (**Figure 1A**).

Seasonality

There was a significant interaction between season, year, region and spider age class (Linear mixed model: $\chi^2 = 43$, $df = 8$,

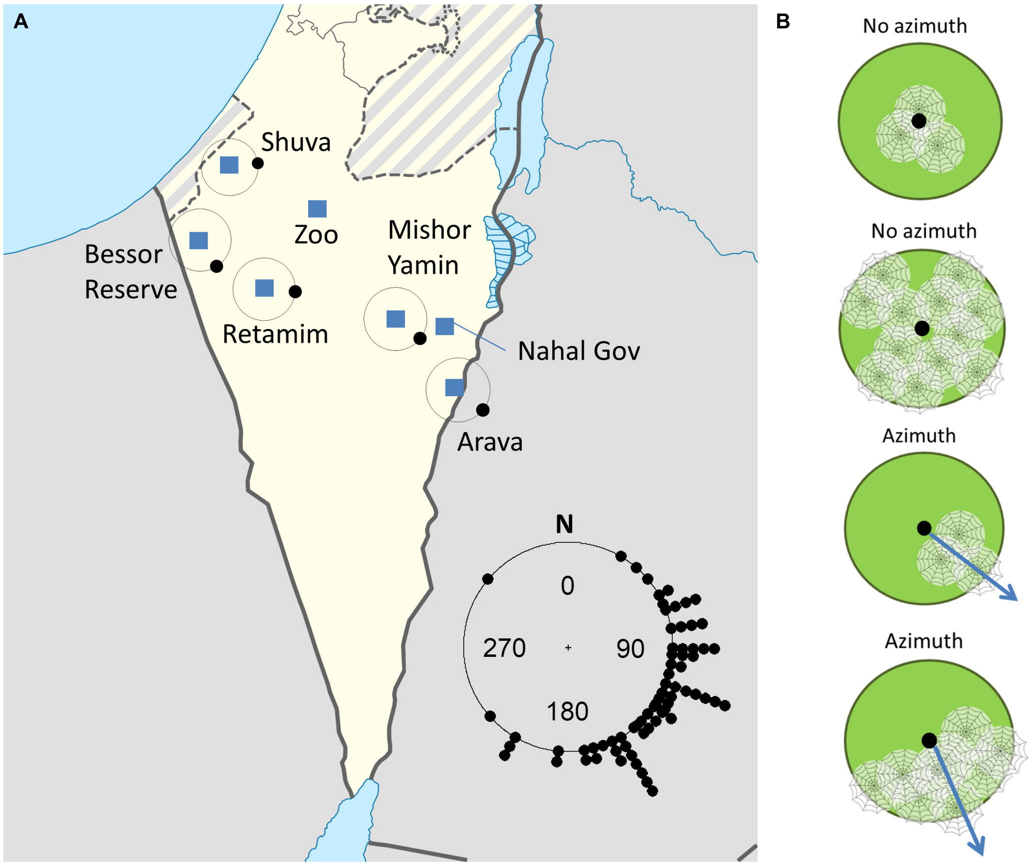

FIGURE 1 | (A) A map indicating the location of census sites (squares) and the average azimuth of colonies at each site (indicated by the black dot on the circle). No colony had a distinct azimuth at the Beer Sheva Zoo (Zoo), and Nahal Gov only had two colonies with distinct azimuths (average 160 degrees off north). The inset circle shows the azimuth of all colonies. **(B)** How azimuth was measured: the green circles represent substrates with the black dot indicating the substrate's center. Colonies (web icons) in the center or occupying the entire substrate had no azimuth. For large colonies that spanned a range of degrees, we took the azimuth (blue arrows) from the middle of the span.

$P < 0.0001$). Colonies were large at the start of observations at the end of 2012 (**Figure 3**), then declined in late winter and early spring of 2013. Colonies increased in size again in the spring and summer of 2014, except in the West Negev. The West Negev decline reflects the sharp population decline at the Bessor Reserve site, possibly due to pesticide application in adjacent citrus groves. Reproductive females peaked in late spring and summer, with the West Negev peaking earlier than the East Negev and Arava regions. These peaks in egg sac production were followed by increases in juvenile populations 2–3 months later (**Figure 3**).

Extinction and Recolonization

Ninety-five colonies (78%) went extinct during the observations, and we observed 66 recolonizations of flagged colonies that had gone extinct. Including these recolonizations, a total of 134 colonies went extinct and 40 survived to the end of the observation period. Even colonies that did not go extinct often experienced a population collapse, with a >90% reduction in population. Out of 174 colonies (including recolonizations), a total of 150 (86%) collapsed or went extinct. When considered alone, both deviation from the preferred azimuth and smaller substrates correlated positively with increased extinction risk (azimuth deviation Cox P.H.: $z = 2.9$, $P = 0.004$; substrate size Cox P.H.: $z = 2.1$, $P = 0.03$). However, the center of larger colonies tended to be closer to the preferred azimuth of the site, and substrate size also correlated with colony size (**Figure 2**) so that these effects disappeared when initial colony size was added to the model. Considered together, initial colony size, region, and substrate type all influenced time to extinction. Larger colonies had a much lower risk of extinction (Cox P.H.: $\chi^2 = 25.2$, df = 1, $P < 0.0001$; **Figure 4A**). Substrate type interacted with region (Cox P.H.: $\chi^2 = 20.5$, df = 1, $P < 0.0001$; **Figure 4B**), which indicated that while colonies on shrubs performed similarly across regions, colonies on *Vachellia* trees survived better in the Arava than in the West Negev (**Figure 4B**). This was because colonies in a row of planted *V. gerrardii* nearly all went extinct at the Retamim site, while most colonies on *V. tortilis* or *V. raddiana* in the Arava region survived.

The rate of recolonization was not affected by substrate size nor by previous colony size (Cox P.H.: substrate size $z = -0.07$, $P = 0.95$; previous colony size $z = 1.02$, $P = 0.31$). When

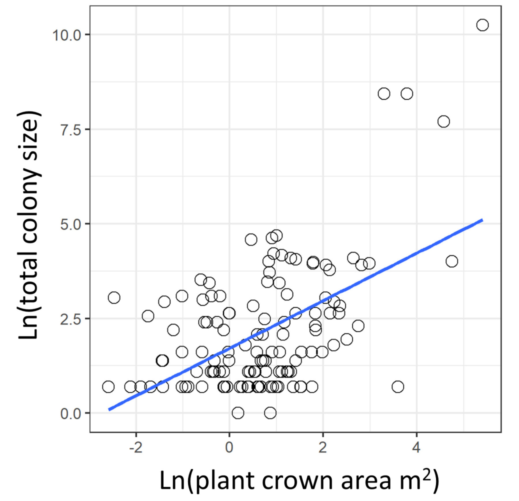

FIGURE 2 | The correlation between substrate size (plant crown area) and the total number of spiders in the colony.

considered by itself, substrate type influenced recolonization rate, with colonies on shrubs being recolonized sooner than those on *Vachellia* (Cox P.H.: $\chi^2 = 10.1$, df = 2, $P = 0.006$). However, this effect disappeared when considered alongside region (Cox P.H.: $\chi^2 = 2.3$, df = 2, $P = 0.32$). Colonies that had gone extinct were more likely to be recolonized in the Arava and East Negev, compared to the West Negev (Cox P.H.: $\chi^2 = 17.4$, df = 2, $P = 0.0002$; **Figure 5A**). This effect was largely driven by the Retamim site, where recolonizations were rare (**Figure 5B**).

Relationships Between Colony Size and Colony Growth

Colonies with fewer females were more likely to either have a high average number of egg sacs per female or no egg sacs at all (**Figure 6**). For only those colonies that produced at least one egg sac, sacs per female decreased with increasing numbers of females (Linear mixed model: $\chi^2 = 33$, df = 1, $P < 0.0001$; **Figure 6B**). When colonies that failed to reproduce are included, sacs per female increased with colony size (Linear mixed model: $\chi^2 = 9.1$, df = 1, $P = 0.003$), and there was a non-significant quadratic effect (Linear mixed model: quadratic term $\chi^2 = 3.5$, df = 1, $P = 0.06$; **Figure 6A**). Similarly, smaller colonies in terms of total number of spiders were more likely to either grow between censuses or go extinct (**Figure 7A**). A significant quadratic effect indicated an optimal intermediate group size of about 185 spiders (Linear mixed model: number of spiders $\chi^2 = 14.6$, df = 1, $P = 0.0001$; number of spiders squared $\chi^2 = 6.1$, df = 1, $P = 0.013$; **Figure 7A**). There was no interaction between colony size and study site, indicating that the humped relationship was consistent across sites (Linear mixed model: number of spiders*site $\chi^2 = 10.3$, df = 10, P = 0.42; number of spiders squared*site $\chi^2 = 9.1$, df = 10, $P = 0.52$). Over the two-year study, spider populations declined overall, so even at the optimal group size, the average colony decreased by 34% from its previous size at the next census. Most colonies that grew from one census to the next were small to medium sized (**Figure 7A**).

Although we detected a positive relationship between the percent increase and colony size in the previous census (i.e., an Allee effect) at the colony level (**Figure 7A**), we did not detect a population-level Allee effect (**Figure 7B**). Instead, population growth in the next census monotonically decreased with population size (Linear mixed model: $\chi^2 = 5.3$, df = 1, $P = 0.02$).

Variation in egg sac production also peaked at intermediate colony sizes (**Figure 8**). The largest single chain of egg sacs (a single chain represents the reproductive output of a single female) was 9 sacs. Only two singletons produced chains of 5, and only one singleton produced a chain of 6. All chains larger than 6 were produced by females living in multi-female groups. The pattern was consistent regardless of whether we examined all large females, some of which may not have reached adulthood (Linear mixed model: number of spiders $\chi^2 = 31.9$, df = 1, $P < 0.0001$; number of spiders squared $\chi^2 = 16.4$, df = 1, $P < 0.0001$; **Figure 8A**), or only those females guarding egg sacs (Linear mixed model: number of spiders $\chi^2 = 83.2$, df = 1, $P < 0.0001$; number of spiders squared $\chi^2 = 28.5$, df = 1, $P < 0.0001$; **Figure 8B**).

DISCUSSION

We examined changes in colony demographics over two years in the colonial spider *C. citricola* to understand how colony size relates to fitness and population growth. Colony orientation largely corresponded to the lee side of the substrate, as winds come off the Mediterranean moving east and then head more toward the south in the Arava Valley (Shemer, 1986). The exception was the Bessor Reserve (**Figure 1A**), which is in a river valley that runs north to south. Reproduction declined in winter in all regions, but reproduction peaked earlier in western populations compared to the Eastern Negev and Arava Valley (**Figures 1A, 3**). In other colonial spider species, wetter habitats are linked to faster development (Fernández Campón, 2010), which may also be the case here. Although there were seasonal cycles of growth and decline, overall spider populations decreased over our observations, with frequent colony extinctions and population crashes. Large colony size could buffer against colony extinction, but otherwise extinction and recolonization probabilities were idiosyncratic to particular sites. Even though single spiders and small colonies could have high growth and reproduction rates, the high incidence of extinction and total reproductive failure depressed average growth and reproduction below that of larger colonies (**Figures 6A, 7A**).

Growth peaked at intermediate colony sizes, but the relationship between colony growth and spider fitness is not necessarily simple. Because *C. citricola* juveniles disperse (Johannesen et al., 2012; Yip et al., 2019), colony size decline could result from a combination of emigration out of the colony and mortality. Over the course of our observations, we added new colonies to censuses. Although some quite large colonies experienced severe population declines, we did not

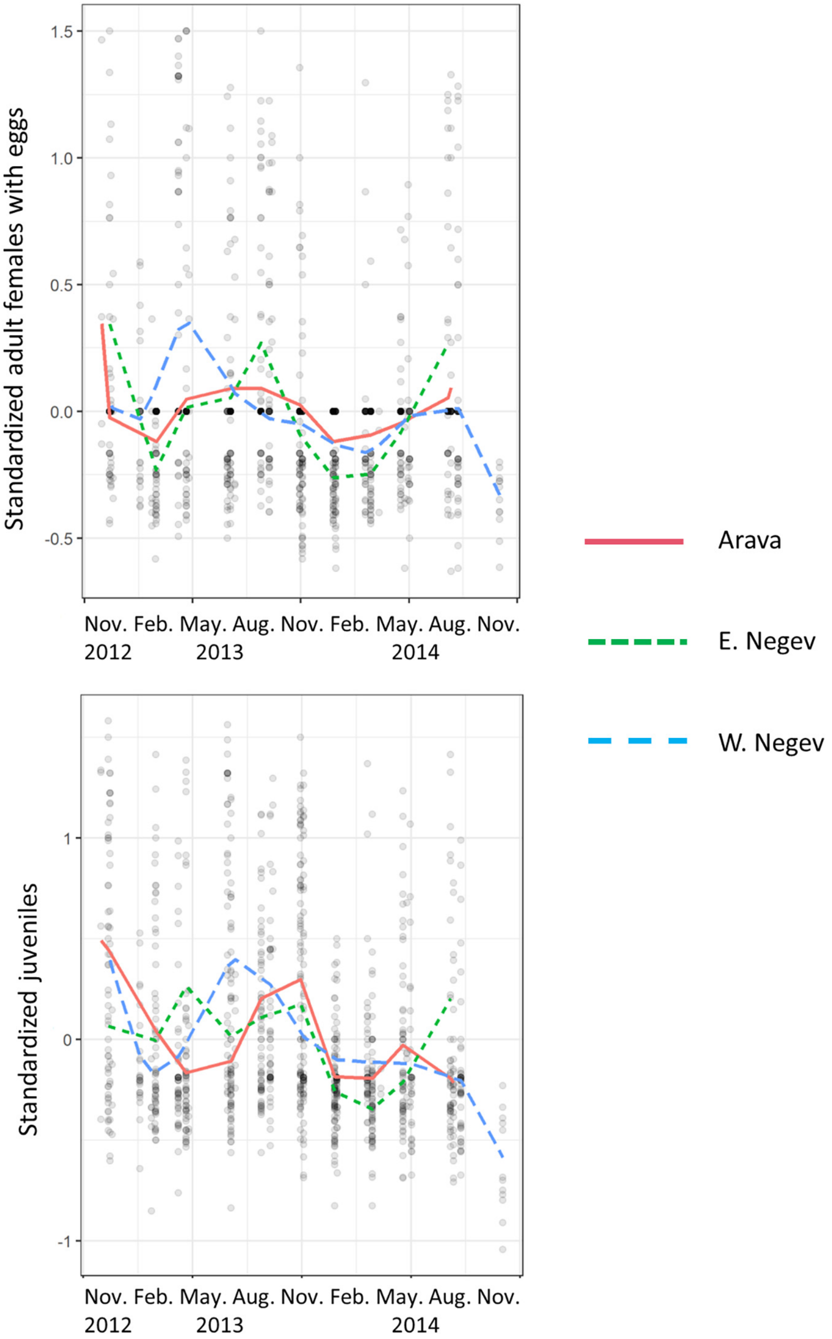

FIGURE 3 | Seasonal fluctuations in standardized colony size, separated by region and spider age class (adult females with eggs and juveniles). Curves are uniform splines of 8 knots.

find very large numbers of new webs or colonies and only 66 recolonizations, suggesting that emigration alone cannot account for colony declines. Undoubtedly, some spiders dispersed outside the study area and could not be counted. However, dispersal is very costly, and net house data show that young spiders that disperse usually die before even building a web (Yip et al., 2019). Furthermore, the pattern of colony growth in relation to colony size (**Figure 7A**) resembled the pattern for egg sac production (**Figure 6A**), suggesting that colony decline is a consequence of low reproductive rates and therefore low individual fitness. Additionally, large colonies might attract more egg parasites (Uetz and Hieber, 1997), so that egg sac number might be a misleading measure of fitness; however, only 20 of 724 (2.8%) collected egg sacs showed signs of parasitism or predation (see **Supplementary Table 3**), suggesting that parasitism was not strong enough at these sites to either explain the decline in colony

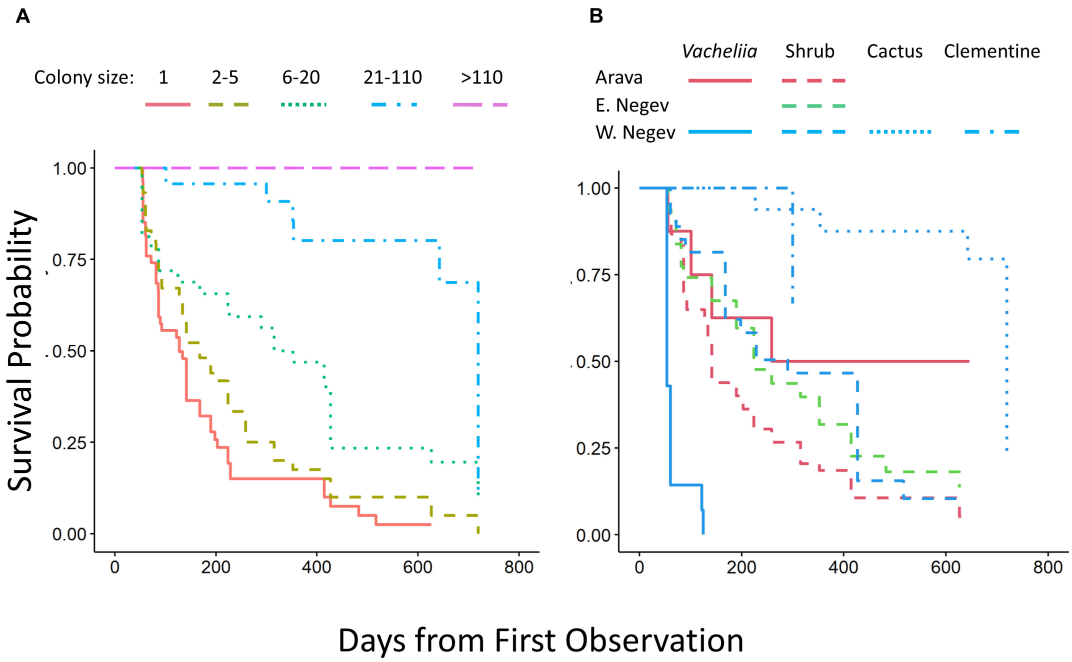

FIGURE 4 | Kaplan-Meier survival curves for *C. citricola* colonies by initial colony size **(A)** and by substrate and region **(B)**.

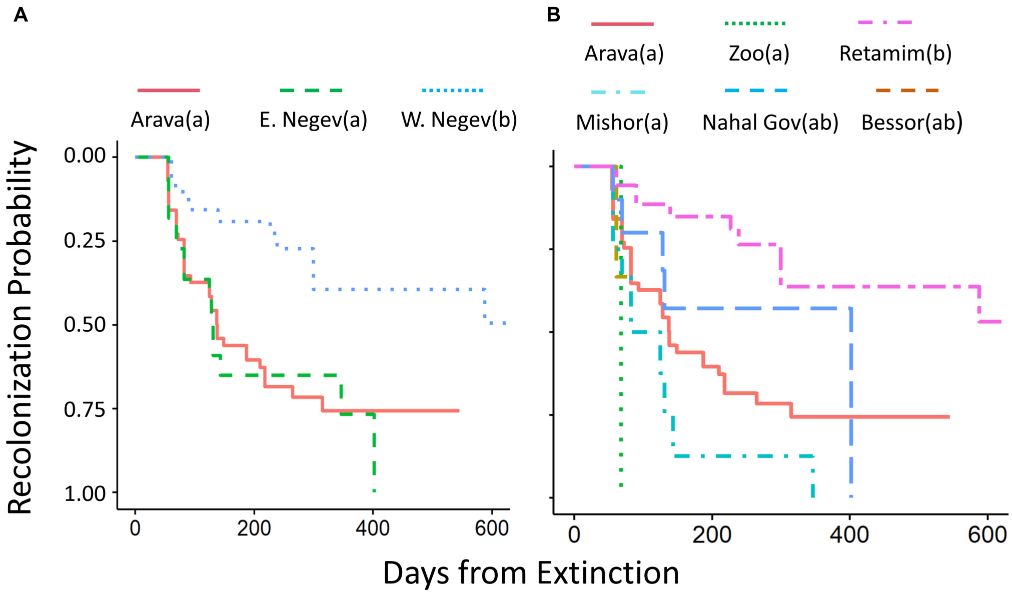

FIGURE 5 | Cumulative recolonization probability by region **(A)** and site **(B)** versus the time elapsed (days) from extinction of the previous colony. Significant differences among curves are denoted by different letters in parentheses in the line legend. Pesticide application caused a population collapse at Shuva, and we ended observations at that site before any colonies went extinct.

growth at the largest sizes or negate the increase in reproduction (egg sac counts) in mid- to large-sized colonies.

Mid-sized colonies (and perhaps larger colonies; see a quadratic effect **Figure 6A**) had a higher number of eggs sacs per female, as well as higher variance in egg sac production (**Figure 8**). Our methods provide snapshots in time, so variation in egg sac production might reflect either differences in reproductive output (and thus fitness) or differences in timing of development, with younger females having not yet reproduced to the same extent as older females. However, we repeatedly observed sites over time, and it is unlikely that mid-sized colonies would consistently have females further

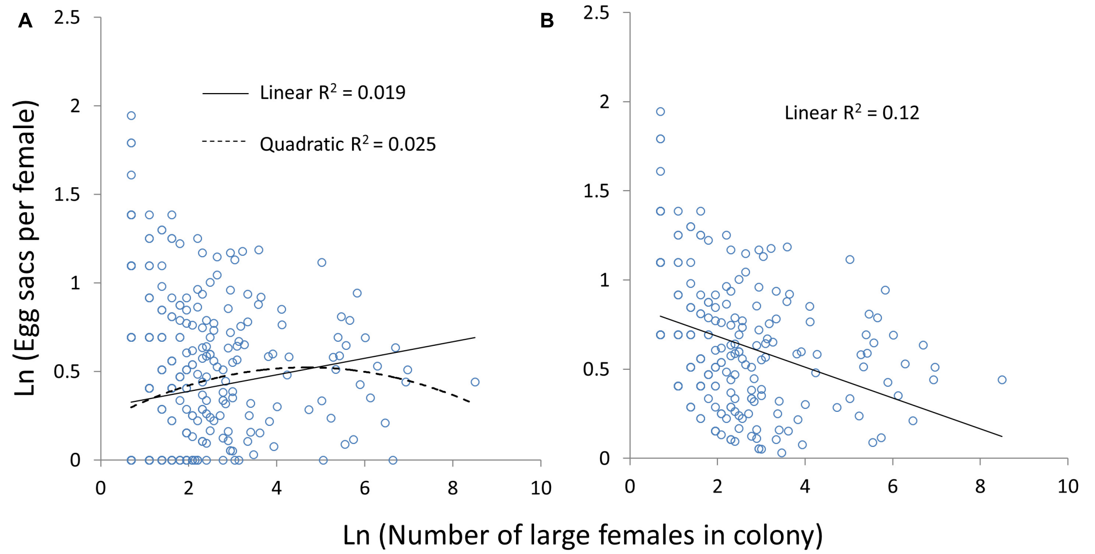

FIGURE 6 | The correlation between the number of adult or subadult females in the colony and the number of egg sacs per female. All colonies are included in panel **(A)**, while colonies that failed to reproduce at all are excluded in panel **(B)**. Significant linear correlations are indicated by solid lines, while a non-significant quadratic effect is indicated by a dashed line.

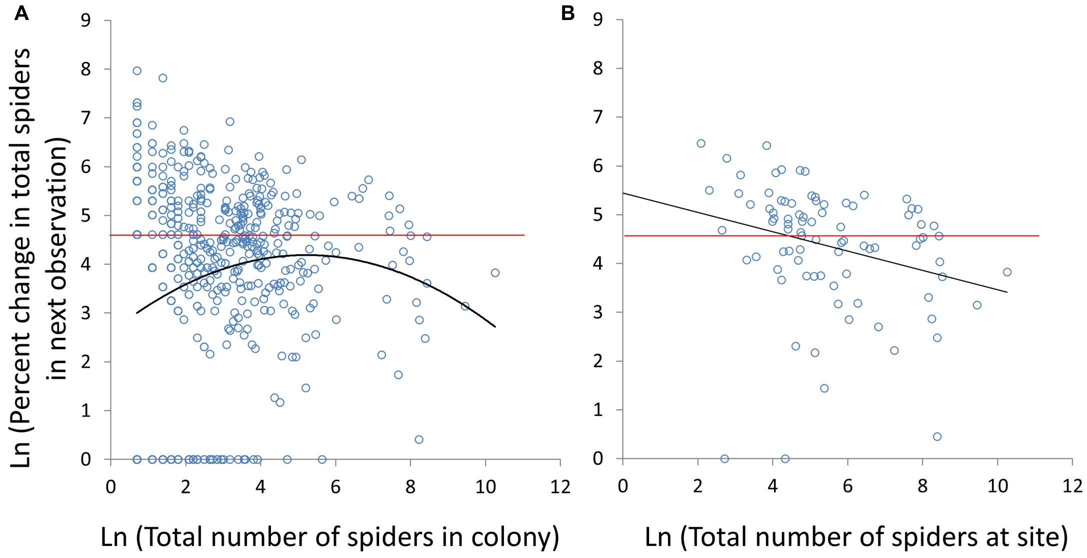

FIGURE 7 | The relationship between numbers of spiders in the colony **(A)** or population (i.e., at a particular site) **(B)** and the percent change in number in the next census. Significant quadratic and linear effects are sown in solid black lines. No change in colony or population size (Log_e 100% = 4.6) is shown with a red line.

along in their reproductive lives than females in larger or smaller colonies. Thus, egg sac variation among colonies likely represents differences in reproductive output. Similarly, within colonies, variation in egg sac production might be due to either asynchronous development of individuals or to variation in reproductive capacity. In either case, increased variation in mid-sized colonies would result from increased variation in prey consumption. Reduced feeding slows development and reduces total reproductive output in *C. citricola* (Yip and Lubin, 2016), and because all spiders in a colony – and moreover an entire field site—experience similar climatic conditions, nutritional differences are the most likely source of increased variation in development over the underlying level of population asynchrony (i.e., spiders being born at different times).

Thus, it seems likely that average fitness does peak at intermediate colony sizes. This pattern is similar to that seen in cooperative social spiders, such as *Anelosimus eximius* and *Stegodyphus dumicola*. In these species, fitness increases with colony size due to very low survivorship of singletons and small groups (Avilés and Tufino, 1998; Bilde et al., 2007). Singletons

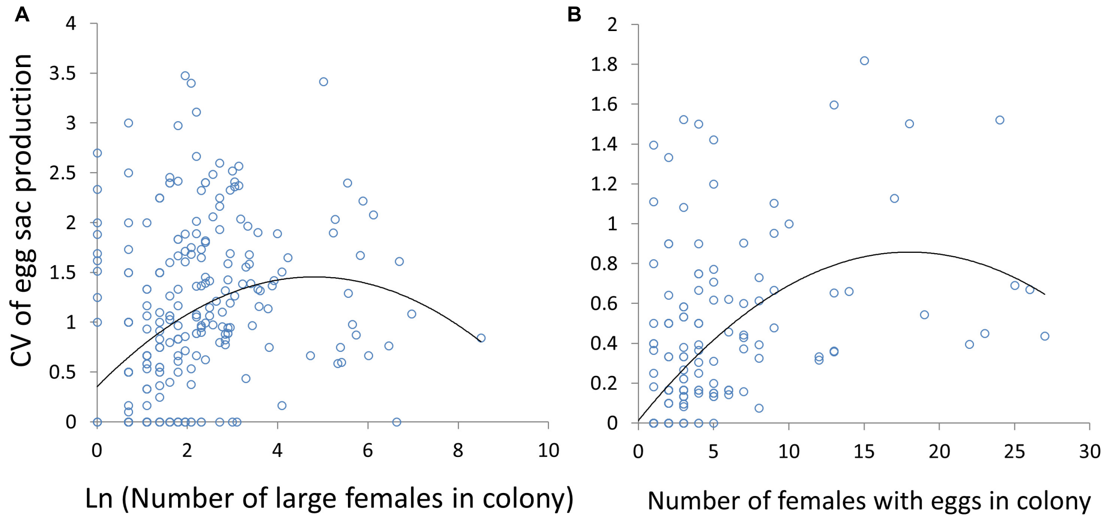

FIGURE 8 | The relationship between the coefficient of variation (CV) of egg sac production within colonies and colony size, measured as the total number of large females in panel **(A)** and only females with egg sacs **(B)**. CVs for singletons were derived from all singletons within a site. Significant quadratic effects are shown in solid black lines.

and small groups could have high reproductive success, but this was offset by poor survivorship, and increased competition in the largest colonies resulted in a humped fitness curve (Avilés and Tufino, 1998; Bilde et al., 2007). Similar patterns were found in subsocial *Anelosimus studiosus*, where small groups of siblings captured more prey per capita than singletons or larger groups (Jones and Parker, 2000). It seems logical that fitness peaks at intermediate group sizes should be universal, as competition eventually overwhelms the benefits of group living (e.g., VanderWaal et al., 2009; Markham et al., 2015), yet surprisingly this is not always borne out by empirical data (Dornhaus et al., 2012; Rudolph et al., 2019). A review of colony size in social insects found that the relationship between group size and reproductive output could be positive, negative, or neutral, with no detectable intermediate peak (Dornhaus et al., 2012). Nevertheless, our data suggest that the general patterns of high fitness at intermediate groups sizes can apply to vastly different spider societies, exhibiting either highly cooperative behaviors (e.g., *A. eximius*, *A. studiosus*, and *S. dumicola*) or mostly passive benefits to group living in colonial species (e.g., *C. citricola*).

Reproductive variation increased with colony size and either decreased or leveled-off at the largest colony sizes, depending on whether larger females without egg sacs were included in the analysis (**Figure 8**). This supports our hypothesis that individuals that anticipate poor reproductive payoffs might leave groups, even if the group has a high average reproductive output. Previous work on *C. citricola* has demonstrated that conflict and dominance hierarchies are common within colonies. Larger spiders typically win interactions and force smaller spiders into less favorable web positions or prevent web construction altogether (Yip et al., 2017). Spiders may also attempt to usurp existing webs (Rypstra, 1979) or steal prey from smaller spiders (E.C.Y. pers. obs.). Because colony webs are constrained by their substrate (**Figure 2**), as colonies grow there may be fewer suitable sites for web construction. In larger colonies, subordinate spiders may have difficulty securing favorable web locations or avoiding aggression by larger neighbors. This conflict may then result in increased variation in prey consumption and weight (Ventura et al., 2017), which may then lead to variation in egg sac production (Yip and Lubin, 2016).

Despite the ability to freely disperse, join neighboring groups, or attempt to survive solitarily (Yip et al., 2019), many females in larger colonies either failed to reproduce or produced only one egg sac, while some of their neighbors produced many egg sacs. High variance in reproduction is also observed in cooperatively social spider species, but these species also form highly inbred demes so that average colony fitness likely also reflects individual inclusive fitness (Avilés, 1997). By contrast, *C. citricola* is outbred with colonies comprising mixtures of kin and non-kin (Johannesen et al., 2012; Yip et al., 2019). Why did *C. citricola* females with low reproduction stay in the colony? One possible answer is that spiders probably have limited information about their future reproductive prospects. Interestingly, when partial information has been incorporated into reproductive skew models, this has increased independent reproduction away from the groups (Kokko, 2003; Akçay et al., 2012), even when staying might have mutually benefited both dominants and subordinates (Akçay et al., 2012). Neither of these models predicted that limited information would increase grouping (Kokko, 2003; Akçay et al., 2012). These models incorporated uncertainty in the amount of reproduction allotted to the subordinate (Kokko, 2003) or prospects away from the group (Akçay et al., 2012). We hypothesize that *C. citricola* spiders might face a different kind of uncertainty. *Cyrtophora citricola* spiders appear to disperse as juveniles (or adult males), while subadult and adult females are largely sedentary (Johannesen et al., 2012; Yip et al., 2019). Dominance in *C. citricola* spiders, as in many species, is related to size, and spiders may face uncertainly about their final size and dominance (Yip et al., 2017). This seems particularly likely because reduced feeding decreases size at a given molt, but spiders can also increase the number molts to adulthood. Thus, juveniles with poor feeding rates might still obtain large adult sizes (Yip and Lubin, 2016). This uncertainty in dominance may be similar to pleometrosis in ants, where multiple queens found a colony, but only one survives to reap the benefits (Tschinkel and Howard, 1983; Teggers et al., 2021). Queens may be uncertain whether they will be the surviving monarch, and by the time they have reliable information about their chances of success (such as fecundity), it is too late to disperse and live solitarily (Teggers et al., 2021).

While we detected an Allee effect at the level of the colony, we did not detect any Allee affect at the population level. It seems paradoxical that Allee effects at the group level do not translate to the population, and some have suggested that the two levels should mirror each other (Courchamp et al., 2000). However, a recent model predicted a strong correlation between group and population Allee effects only if group size homogeneity was moderate to high (Angulo et al., 2018). Group sizes can be extremely heterogeneous in *C. citricola* populations, with large colonies surrounded by smaller groups and singletons (**Supplementary Figure 1**). The mismatch between colony and population Allee effects supports the model's assumptions that larger groups can buffer Allee effects in smaller groups at the population level (Angulo et al., 2013, 2018).

Interestingly, we found a strong negative correlation between population size and growth in the next census (**Figure 7B**). In African wild dogs, there is a great deal of group size heterogeneity and groups largely avoid one another, so that there was no relationship between population size and growth rate (Angulo et al., 2013). Because *C. citricola* colonies are physically attached to their substrates, they do not interact directly, and so we predicted similar independence of population size and growth. The negative relationship could stem from large colonies with poor growth (**Figure 7A**) driving the dynamics of the overall population. This may explain why *C. citricola* has been a successful invader despite having higher fitness at intermediate group sizes. If a few founders can survive small colony size (which is not uncommon; see **Figures 4A**, **7A**), the incipient population is likely to do well without large colonies of competing conspecifics.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

EY and YL collected and analyzed the data. All authors conceived and designed the project, helped secure funding for the project, and contributed to writing 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.2021.725647/full#supplementary-material

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Non-kin Cooperation in Ants

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Eusociality represents an extreme form of social behavior characterized by a reproductive division of labor. Eusociality necessarily evolved through kin selection, which requires interactions among related individuals. However, many eusocial taxa also show cooperation between non-kin groups, challenging the idea that cooperative actions should only occur among relatives. This review explores the causes and consequences of non-kin cooperation in ants. Ants display a diversity of behaviors that lead to non-kin cooperation within and between species. These interactions occur among both reproductive and non-reproductive individuals. The proximate and ultimate mechanisms leading to non-kin cooperative interactions differ substantially depending on the biotic and abiotic environment. We end this review with directions for future research and suggest that the investigation of non-kin cooperative actions provides insight into processes leading to social evolution.

Keywords: conflict, cooperation, haplometrosis, parabiosis, mutualism, polygyny, pleometrosis, foundress associations

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INTRODUCTION

Cooperation is a fundamental part of life and occurs among entities at all levels of biological organization (Maynard Smith and Szathmary, 1998). A general evolutionary definition of cooperation is a behavior which benefits another individual, and which has been selected for because of its positive effects on both participants (after West et al., 2007b). Thus, the focal social behaviors must have evolved at least partially because of the fitness benefits that they produce (West et al., 2007b). These fitness benefits can be either direct or indirect. Direct fitness benefits refer to gains in a focal individual's own reproductive success. Indirect fitness benefits result from increased reproductive success for relatives of the cooperating individual (Hamilton, 1964).

Cooperation within species of eusocial insects usually occurs between relatives. That is, the stereotypical lifestyle for a eusocial insect colony is that of a cooperative, family group (Hölldobler and Wilson, 1990; Ross and Matthews, 1991; Hughes et al., 2008). A "standard" eusocial hymenopteran colony is often headed by a single queen who produces the worker offspring that cooperate to build the nest, rear the young, forage, etc. The workers do not gain direct benefits for such actions, since they are (more or less) sterile. Instead, they receive indirect benefits by cooperating because they rear related offspring that will pass on their genes. This familial system of cooperation and reproductive altruism can evolve because the nestmates are related. If they were not, then such cooperative systems with reproductive altruists (e.g., sterile workers) could not evolve (Kay et al., 2020).

Surprisingly, eusocial insects sometimes engage in non-kin cooperative behaviors (Jackson, 2007; Helantera et al., 2009; Leniaud et al., 2009; Lehmann and Rousset, 2010; Moffett, 2012; Boomsma and d'Ettorre, 2013; Hakala et al., 2020; Ostwald et al., 2021). Such actions are unexpected because eusocial insects are the paradigm of kin cooperative actions. Nevertheless, cooperative behaviors among non-kin occur in several different contexts. Such interactions require careful examination and explanations, because they would seem to contradict traditional models of cooperation in these taxa.

The purpose of this review is to examine non-kin cooperative behaviors in ants. We define non-kin here as associations where relatedness is low (e.g., zero or near zero), and consequently there are little to no indirect benefits from helping relatives. We discuss cases were non-kin cooperation may occur within species. Such situations encompass most well-studied and well-known examples of intraspecific cooperation between non-relatives. In addition, we extend our review to include unusual instances of cooperation among non-kin that occur in species with unusual genetic systems. We also consider cases of *interspecific* behaviors as instances of non-kin cooperative actions. The causes and consequences of interspecific cooperation differ from those for intraspecific cooperation, thereby providing useful points of comparison. Finally, we provide suggestions for areas of future research in non-kin cooperation (West et al., 2021).

Our review specifically focuses on non-kin cooperation in ants. Ants are perhaps the most well-studied eusocial insects in terms of taxonomic breadth (Hölldobler and Wilson, 1990), and diverse examples of non-kin cooperative actions in ants have been identified (**Figure 1**). We discuss several of these examples to understand the proximate causes and ultimate consequences of these cooperative interactions. Overall, the study of non-kin cooperation in ants provides great insight into the evolution of social actions in animal societies.

NON-KIN COOPERATION AMONG ANT QUEENS

Most ant colonies are headed by a single reproductive queen (i.e., monogyne colonies), which is the likely ancestral condition for eusocial Hymenoptera generally (Hughes et al., 2008). However, multiple-queen (polygyne) colonies are common in ants and polygyny has evolved independently in nearly every ant subfamily (Hölldobler and Wilson, 1977; Keller, 1993). However, the exact number of times polygyny has evolved in ants, or the number of species that are polygyne, has not been quantified to our knowledge. Polygyny can arise through a variety of mechanisms including the recruitment of sisters from within the nest, the adoption of unrelated queens from other nests, and from newly mated queens cooperating to start new colonies (Hölldobler and Wilson, 1977; **Figure 2**). In this section, we discuss the ecology and evolution of polygyny, particularly as it relates to associations of non-kin queens. Such non-kin associations actually represent a fundamentally important part of the lifecycle of many ants.

Primary Polygyny

In many ant species, unrelated queens initiate colonies in groups, a process known as pleometrosis (Hölldobler and Wilson, 1977). These associations among reproductives are taxonomically widespread with examples in all four of the largest ant subfamilies (Ponerinae, Myrmicinae, Dolichoderinae, and Formicinae) (Bernasconi and Strassmann, 1999). The proximate mechanisms leading to queen aggregations are not well known and may be diverse. For example, queens may be attracted to the same microhabitats thereby ending up in the same location to establish a new nest (Tschinkel and Howard, 1983). Queens may also form pleometrotic assemblages by searching out other queens as suggested for some populations of the weaver ant, *Oecophylla smaragdina* (Peeters and Andersen, 1989; Crozier et al., 2010). It is even possible that queens locate each other using pheromones or other attractants (Aron and Deneubourg, 2020).

Natural selection will favor traits that lead to pleometrotic associations when the success of independent colony founding (haplometrosis) is very low (Shaffer et al., 2016; Haney and Fewell, 2018); independent colony founding rates are indeed estimated to be less than 1% in many ant species (Hölldobler and Wilson, 1990; Aron and Deneubourg, 2020). The formation of pleometrotic associations would therefore be driven by mutualism; kin selection (i.e., relatedness) would not necessarily play a role. Instead, an individual queen's direct fitness would be higher, on average, by joining a group than if she founded a colony independently. Similarly, groups should allow other queens to join as long as individual fitness increases with queen number and provided that group size does not reach a point of diminishing returns.

There are a number of benefits to pleometrotic associations (Bernasconi and Strassmann, 1999; Ostwald et al., 2021; Teggers et al., 2021), notably the ability to produce workers more quickly than through haplometrosis. Many species that form pleometrotic associations are highly territorial, and workers from established colonies may destroy or raid incipient colonies in their immediate vicinity. Quickly producing a large worker force will therefore increase forging, protect the colony, and increase the success of the focal colony's own raids. In addition, cooperating queens may display division of labor if they vary in their tendency to perform specific behaviors such as excavation (Helms Cahan and Fewell, 2004); by performing tasks such as digging, taking care of brood, and foraging in parallel, overall efficiency is increased during the critical stage of colony founding.

In some cases, pleometrotic associations may lead to permanently polygyne colonies (**Figure 2**). That is, the initial associations of unrelated queens persist through colony ontogeny. The proximate mechanisms leading to such associations have been investigated in some ant taxa (Clark and Fewell, 2014; Helmkampf et al., 2016; Overson et al., 2016; Shaffer et al., 2016; Eriksson et al., 2019; Masoni et al., 2019; Aron and Deneubourg, 2020). Despite the initial benefits of primary polygyny, it is believed to be relatively rare for pleometrotic associations to result in permanent polygyny (Hölldobler and Wilson, 1990; Bernasconi and Strassmann, 1999; Eriksson et al., 2019). While queens exist peacefully at the onset of pleometrotic associations, colony members may no longer tolerate each other after workers emerge. Queens may fight directly leading to a single, surviving reproductive, or queens may compete indirectly during production of their first brood (Teggers et al., 2021). For example, *Solenopsis invicta* fire ant queens vary in weight loss during reproduction in pleometrotic associations. These differences are associated with success as heavy queens are more likely to survive fights (Bernasconi and Keller, 1996;

FIGURE 1 | Examples of ants that exhibit non-kin associations or variation in queen number along a continuum of relatedness. **(A)** *Solenopsis invicta* queens form polygyne colonies consisting of unrelated queens (photo credit: Haolin Zeng). **(B)** *Pogonomyrmex californicus* queens from a polygynous population (photo credit: Elizabeth Cash). **(C)** An association of over 20 *Oecophylla smaragdina* collected in Darwin, Australia from a rolled leaf where they had enclosed themselves (photo credit: Andrew Suarez). **(D)** Trophilaxis between *Camponotus* and *Crematogaster* workers in a parabiotic relationship in Malaysia (photo credit: Florian Menzel).

Bernasconi et al., 1997). Queens in pleometrotic associations of a variety of species will eat each other's eggs. This behavior not only increases their food intake, but also reduces worker production of rivals. Thus, queens may be preparing for fighting even while cooperating to start a new colony. Workers may also take part in the culling of queens, their eggs, or larvae. In *S. invicta*, workers do not treat their mother differently from other unrelated queens within the association, and may directly or indirectly participate in the destruction of their own mother. In *Lasius* and *Messor*, larvae eat eggs but there is no evidence that they can discriminate between related and unrelated eggs (Urbani, 1991). Thus, selection for direct kin helping in pleometrotic associations apparently does not occur. Given the lack of permanent polygyny in most pleometrotic species, a case could be made that these associations could be described as facultative or even competitive rather than cooperative.

Secondary Polygyny

Polygyny in ants typically occurs through secondary adoption of queens as the colony ages (Boomsma et al., 2014; **Figure 2**). Secondary polygyny, therefore, often leads to permanently polygyne nests. The proximate mechanisms leading to secondary polygyny likely differ from those that lead to primary polygyny in pleometrotic associations. However, we know surprisingly little about the proximate mechanisms leading to polygyne colonies and variation in polygyny within and among species. An exception is fire ants in the genus *Solenopsis*, where genetic variation at a large supergene plays a decisive role in the formation of polygyne nests (Krieger and Ross, 2005; Gotzek, 2007; Arsenault et al., 2020; Yan et al., 2020). In this case, workers behave differently toward queens of distinct supergene genotypes; this behavioral variation ultimately only leads to the acceptance of queens possessing heterozygous genotypes in polygyne colonies. The genes within the supergene influence the cues and behaviors used for this genetic discrimination (Fletcher and Blum, 1983) possibly through chemically mediated cues (Eliyahu et al., 2011; Trible and Ross, 2016) leading to an unusual green-beard phenomenon in this species (Keller and Ross, 1998).

The ultimate factors responsible for the evolution of polygyny have been more widely considered than the proximate factors (Keller, 1993). In particular, as with primary polygyny, selection will favor secondary polygyny when having multiple queens enhances colony success (Boulay et al., 2014) or if there is a low likelihood that queens can found colonies independently (Keller, 1991). For example, predation, nest site limitation, intraspecific competition, and nest raiding can select for queens to join established colonies. However, there could be selection against

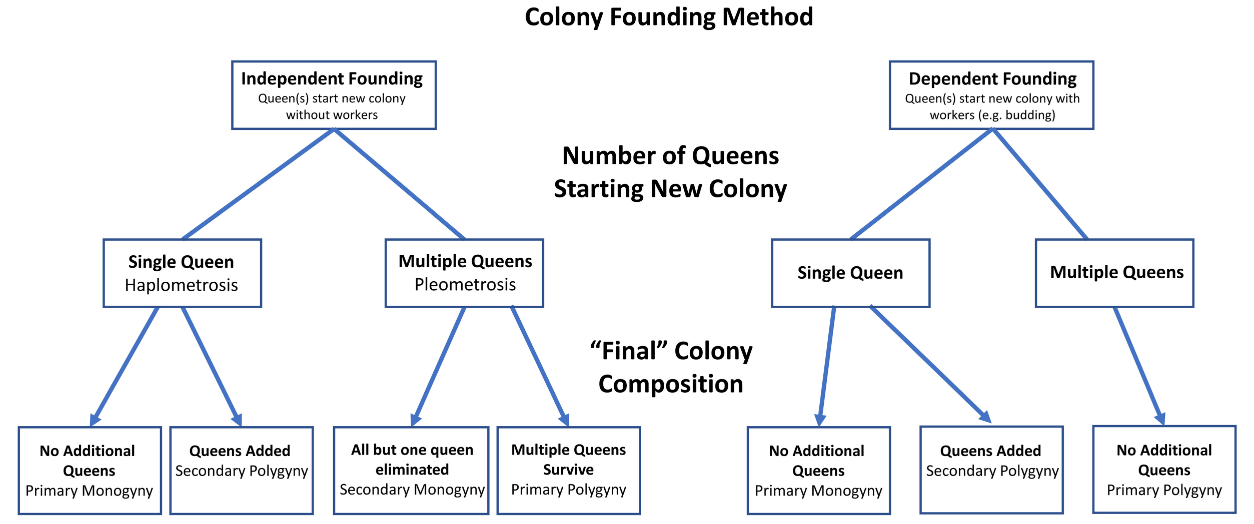

FIGURE 2 | Mechanisms leading to variation in colony-queen number in ants over time. Non-kin queen associations occur when alates from different nests come together to start new colonies (pleometrosis through independent colony founding) or when unrelated queens are adopted into existing colonies (secondary polygyny). Modified from Hölldobler and Wilson (1977).

queens and workers within existing colonies from allowing new queens to join. Polygyny can increase competition for resources and lead to conflict over reproduction among queens; an increase in queen number is often associated with a decrease in individual reproductive output. Moreover, polygyny decreases relatedness among nestmates, which reduces indirect benefits to workers and potentially increases intracolonial conflict. In many ants, polygyny is associated with "budding" reproduction where groups of queens establish new nests accompanied by a large retinue of workers (Cronin et al., 2013; Ellis and Robinson, 2014). Ants that found colonies by budding rather than independently also tend to invest less in the condition of each reproductive, which are subsequently no longer capable of founding colonies without the help of workers (Peeters and Ito, 2001).

It is likely the environment plays a strong role in determining where polygyny can occur and if queen condition restricts independent colony founding (Heinze and Tsuji, 1995; Purcell et al., 2015). Colonies should accept new queens if there is a strong likelihood that a colony will lose its own reproductive. This leads to a prediction that queen adoption, particularly from within the nest, should be more likely as a colony ages or as the current queen(s) condition worsens. Recruitment of new queens will also be selected for if the new queens introduce benefits to the colony such as those associated with increased genetic diversity generally. For example, workers in genetically diverse colonies may be more polymorphic, undertake a greater range of tasks, or better resist disease (Schwander et al., 2005; Smith et al., 2008; Schluns and Crozier, 2009; but see Fournier et al., 2008).

Under most circumstances, however, colonies should only accept relatives as new queens. Queens in most polygyne species are related, indicating that queen recruitment occurs from within the nest (Sundström et al., 2005). However, some ants have colonies that contain unrelated queens, indicating that non-nestmate recruitment occurs (Kummerli and Keller, 2007; Seppa et al., 2012; Sorger et al., 2017; Brodetzki et al., 2020; Hakala et al., 2020). Such associations lead to non-kin cooperation among nestmates and, in these circumstances, would seem to be evolutionarily problematic.

Polygyny is overrepresented in invasive or tramp ant species (Heinze and Tsuji, 1995); in these cases, having large numbers of queens is linked to a variety to mechanisms that likely contribute to colony success (Holway et al., 2002; Boulay et al., 2014; Eyer and Vargo, 2021). For example, polygyny is associated with increased worker production, success of incipient colonies, and probability of transported propagules containing reproductives (Holway et al., 2002; Boulay et al., 2014; Bertelsmeier et al., 2017; Eyer and Vargo, 2021). Introduced species such as the Argentine ant, *Linepithema humile*, and little fire ant, *Wasmannia auropunctata*, can form expansive supercolonies (Giraud et al., 2002; Tsutsui and Suarez, 2003; Foucaud et al., 2009; Helantera et al., 2009). The size of their supercolonies is frequently associated with disturbance, even within native populations, suggesting that introduced ants may provide model systems for understanding widespread cooperation of individuals that are not direct relatives. Introduced *S. invicta* fire ants also form large polygyne colonies that recruit non-nestmate queens (Goodisman and Ross, 1998). In the US, the monogyne social form arrived first but has been replaced with polygyne form indicating some increased success of the polygyne social form under some circumstances.

UNCONVENTIONAL GENETIC SYSTEMS AND NON-KIN COOPERATION IN ANTS

An unusual form of non-kin cooperation has been found in some ant taxa that possess non-standard genetic and reproductive systems (Fournier et al., 2005; Ohkawara et al., 2006;

Foucaud et al., 2007; Pearcy et al., 2011; Kronauer et al., 2012; Eyer et al., 2013; Rabeling and Kronauer, 2013; Okamoto et al., 2015; Lacy et al., 2019). For example, the longhorn crazy ant, *Paratrechina longicornis*, displays an unusual genetic system that leads to cooperative behaviors among "non-relatives" (Pearcy et al., 2011). Workers are produced through standard sexual reproduction between queens and males. However, queens are produced clonally and are genetically identical to their mothers. Strangely, males are also produced in a pseudoclonal fashion and are genetically identical to their fathers (i.e., androgenesis) (Goudie and Oldroyd, 2018). The mechanisms leading to androgenesis may be diverse (Goudie and Oldroyd, 2018). But research suggests that males may be derived from the elimination of the queen genome from diploid eggs or from fertilization of eggs lacking the queen genome altogether (Fournier et al., 2005; Foucaud et al., 2007, 2010; Schwander and Oldroyd, 2016).

The long-term consequence of ant species with odd genetic systems is that queens, males, and workers within colonies show substantial genetic differences. Workers are more closely related to workers from other colonies than they are to their parents or to their reproductive gyne and male "siblings" from their own colony (**Figure 3**). The relatedness dynamics are a bit awkward in these systems. Nevertheless, these do represent an instance of non-kin cooperation between the genetically differentiated worker, queen, and male castes within colonies.

An increasing number of non-standard genetic systems have been identified in ants in recent years (Eyer et al., 2019; Lacy et al., 2019). Therefore, it is possible that instances of this type of non-kin cooperation is even more common than expected. Non-standard reproductive systems may have evolved because they prevented certain types of inbreeding. Such systems also potentially lead to coadapted gene complexes within castes. However, the ultimate fate of species displaying these unusual genetic systems remains unclear.

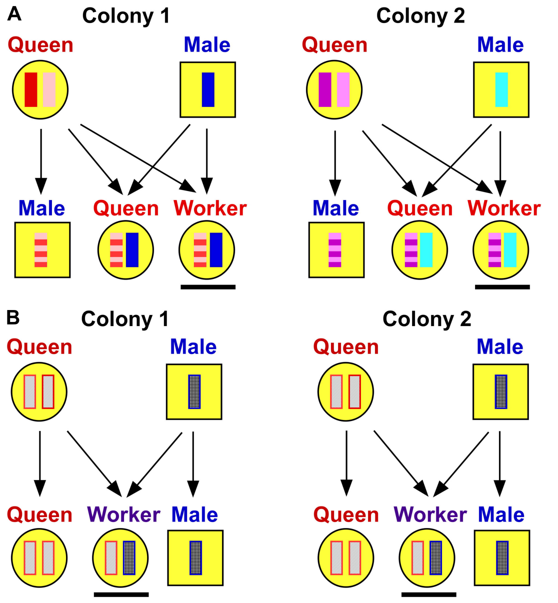

FIGURE 3 | The effects of "standard" and "non-standard" genetic systems on relatedness in ants. **(A)** Genetics of a standard, monogynous, monandrous ant. Haplodiploid genetics lead to relatively high relatedness of 0.75 between female offspring within colonies whereas non-colonymates, such as the underlined workers, are effectively unrelated. Therefore, individuals are expected to engage in cooperative behaviors colonymates who are kin. **(B)** Unorthodox genetic system found in some ants leads to unusual relationships. In the case of the long-horned crazy ant, *Paratrechina longicornis*, for example, underlined workers are produced by "hybridization" of genetically differentiated queens and males, whereas queens and males are effectively cloned. The consequence of this system is that workers in one colony are ultimately genetically identical to workers in other colonies. Moreover, workers are unrelated to their male and queen "siblings" within colonies. The consequences of this type of genetic system on the evolution of cooperative behaviors are unclear.

INTERSPECIFIC ASSOCIATIONS IN ANTS

Research on non-kin associations in ants primarily focuses on intraspecific unions of queens. However, remarkably, cooperative associations between ants of *different* species also occur. For example, associative behaviors such as trail sharing (Wilson, 1965; Davidson, 1988) and nest sharing (i.e., parabiosis) (Davidson, 1988; Orivel et al., 1997; Errard et al., 2003; Sanhudo et al., 2008) can take place between species.

The evolutionary factors that lead to interspecific associations and cooperation between worker ants may be broadly similar to those that lead to non-kin interactions within species (Barker et al., 2017). That is, kin selection cannot be involved because the interactors are not related (West et al., 2011). Instead, both cooperating species must gain some direct benefits so the cooperative action is mutually beneficial (West et al., 2007a). Under this framework a variety of cooperative associations are possible.

Ants engaged in parabiotic associations cooperate in a variety of ways including shared nest defense, trophallaxis, and communal use of trail pheromones. For example, "ant gardens" in the Neotropics (Davidson, 1988; Orivel et al., 1997) and in Asian rainforests (Kaufmann and Maschwitz, 2006; Menzel and Bluthgen, 2010) are often co-inhabited by species from the genera *Crematogaster* and *Camponotus* (along with a number of other genera). These associations start when ants collect seeds of specialized epiphytes or other plants, and incorporate them into carton or soil where the plants grow to increase the size and stability of the ant nest. While brood chambers are kept separate, the rest of the colonies mix freely within the joint nest. In addition to sharing foraging trails to plant-based resources (Menzel et al., 2010), both species will defend the nest although larger *Camponotus* tend to exhibit the majority of the defensive behaviors (Menzel and Bluthgen, 2010).

Fungus growing ants also exhibit a variety of parabiotic associations (Sanhudo et al., 2008; Adams et al., 2013). For example, *Megalomyrmex* "guest ants" were originally thought to be social parasites of fungus growing ants due to their negative effect on host colony growth and garden biomass (Adams et al., 2012). However, *Megalomyrmex* ants apparently provide some benefits because they prevent raids by the genus *Gnamptogenys*, which are specialized agro-predators and are a high source of mortality to *Trachymyrmex* and *Sericomyrmex* fungus growing ants (Dijkstra and Boomsma, 2003). *Megalomyrmex* patrol the nests of their host and prevent raids by producing an alkaloid venom that is both highly toxic to the raiders as well as disrupts their nestmate recognition system (Adams et al., 2013).

Parabiotic associations are interesting as they highlight beneficial consequences of having an environmental or learned, as opposed to strictly "genetic," nest-mate recognition system (Menzel et al., 2008; Emery, 2013; Neupert et al., 2018). They are also fascinating models to study the context dependent nature of species interactions (e.g., parasitism vs. cooperation) (Adams et al., 2013; Menzel et al., 2014). As with queen associations, parabiotic and plesiobiotic associations may be driven by nest site limitation (Kanizsai et al., 2013), although more work is needed to understand how and why these associations evolve.

FUTURE DIRECTIONS

A great deal of important research has been conducted in the study of non-kin cooperation in ants. For example, the use of genetic markers has given us insight into the general frequency and distribution of non-kin cooperative activities. Experimental work has yielded important information on the behaviors that govern non-kin cooperation in some settings. Theoretical discussions have also provided insight into the processes that govern the evolution of cooperative interactions. However, despite these advances, there is still a great deal that remains to be understood about non-kin cooperation in ants. Below, we outline eight areas that should be a priority for future study.

1. *Proximate mechanisms*: Additional research is needed on understanding the proximate mechanisms involved in non-kin cooperation. What sensory modalities operate in non-kin interactions? How do these modalities function? What cognitive processes occur during kin vs. non-kin cooperation and discrimination? How are these processes shaped by developmental factors including experience?
2. *Invasive ants*: Native and introduced populations of ants often display major differences in social structure, with many invasive ants showing non-kin cooperation. However, more research is needed on understanding patterns of cooperation in native vs. invasive ants. Indeed, for many species, we have yet to identify the native source of introduced populations to make such comparisons. We still need fundamental information such as the relatedness of queens and workers in native populations. We also need to learn if changes in social structure are the consequence of increased costs associated with colony founding or whether they result from genetic changes associated with the invasion process.
3. *Cheating*: Evolutionary theory suggests that many types of non-kin cooperation should be susceptible to cheating. For example, selection should favor the ability of workers to discriminate between related vs. unrelated queens. So, can we determine if cheating occurs? Does non-kin cooperation involve enforcement mechanisms or conflict? Can we identify and experimentally manipulate recognition cues such as cuticular hydrocarbons to "cause" nepotistic interactions? We also need to learn what genetic systems underlie recognition processes. And, if environmental nest mate recognition is susceptible to cheating, why have genetic-based nest-mate recognition systems not evolved?
3. *Social parasitism:* The presence of unrelated queens within ant nests sets up potentially interesting dynamics. For example, unrelated new queens could be viewed as social parasites within the colony. Thus, the evolutionary persistence of such colonies represents somewhat of a puzzle. Can polygyny select for variation in queen morphology (e.g., ergatoids queens)? Is queen polymorphism a first step toward parasitism? Can genetic changes be identified that are associated with parasitic behaviors?
4. *Variation in queen number*: Many ants, such as some species in the genera *Formica*, *Leptothorax*, *Linepithema*, *Myrmica*, and *Solenopsis*, are polygyne for part of their life cycle, or show variation in queen number among colonies or seasonally within colonies. What determines how many queens a colony has? What are the proximate mechanisms involved in determining queen number? And does colony queen number actually match evolutionary expectations?
5. *Parabiosis*: Interspecific cooperative actions between ant species is of considerable interest. Indeed, parabiosis may be common, but is understudied. We need more research aimed at understanding how often interspecific ant cooperation occurs. What types of cooperative interactions occur between species? What are the fitness consequences? How often do the interactions change from cooperative to parasitic?
6. *Genetics*: Mapping phenotype to genotype has been a core goal for many evolutionary biologists. But understanding how genetic variation leads to behavioral variation is difficult. Future studies should seek to understand how genetic variation is linked to variation in cooperative actions. For example, recently, "supergenes"—large non-recombining regions of the genome– have been found to be involved in social evolution in a variety of taxa. Do such supergenes underlie certain types of polygyny and lead to non-kin cooperation? If so, what genes are involved in these behaviors and how do they evolve?
7. *Environment:* Theory has provided abundant explanations for how environmental variation should affect non-kin cooperation. However, we have a poor understanding of how and when the environment selects for pleometrotic associations. Thus, more experimental research is needed to understand exactly what environmental conditions lead to cooperative actions.
8. *Distribution/Phylogeny*: Non-kin cooperation is patchily

distributed. So why does non-kin cooperation occur in some species but not others? What evolutionary pressures differ in these cases? And what proximate mechanisms allow non-kin cooperation to occur in only some taxa?

AUTHOR CONTRIBUTIONS

Both authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Competition Drives Group Formation and Reduces Within Nest Relatedness in a Facultatively Social Carpenter Bee

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Animals respond to competition among kin for critical breeding resources in two ways: avoidance of direct fitness costs via dispersal of siblings to breed separately, and formation of kin-based societies in which subordinates offset direct fitness costs of breeding competition via altruism and increased indirect fitness. In the facultatively social eastern carpenter bee, nests are a critical breeding resource in perpetually short supply, leading to strong competition among females. Observations of individually marked and genotyped females in conditions of high and low resource competition demonstrate that competition leads to resource sharing and group nesting. However, in contrast to almost all known animal societies, females avoid nesting with relatives, and disperse from their natal nests to join social groups of non-relatives. This is the first example of a structured insect society with cooperation nestmates, the majority of which are unrelated; thus cooperation is more likely based on selection for direct, rather than indirect fitness. By forming social groups of non-kin, females avoid the indirect fitness costs of kin competition among sisters, yet increase their chances of successful reproduction, and thus direct fitness, when forming colonies of non-relatives.

Keywords: social evolution, *Xylocopa virginica*, nesting resources, behavioral plasticity, bee

INTRODUCTION

Ultimately, the evolution of social behavior in animals is shaped by competition for crucial resources linked to reproduction, including such things as food or breeding sites. One way for animals to improve their access to breeding resources is by forming coalitions or groups of cooperative individuals that work together to obtain and share resources. Cooperative and helping behaviors are a major reason why groups of individuals can have higher per-capita fitness than solitary individuals (Rubenstein and Abbot, 2017). However, group-living does not shield animals from resource competition: dominant individuals often take proportionally greater shares of crucial breeding resources and get more breeding opportunities than subordinates (Ridley and Sutherland, 2002; Bach et al., 2006; Johnstone, 2008). As a result, while dominant individuals living groups may have significantly higher inclusive fitness than individuals breeding alone, the opposite may be true for non-breeding subordinates, whose inclusive fitness is lower than it would be if they bred independently (Richards et al., 2005). In social insects, subordinate females have several options that potentially improve their inclusive fitness by leaving their natal group to breed elsewhere: they can wait for opportunities to replace dominant reproductives (e.g., replacement queens in eusocial sweat bees; Awde and Richards, 2018), they can leave the natal nest and found their own nests elsewhere (Schwarz et al., 2007), or they can join a different breeding group which offers better reproductive opportunities (Leadbeater et al., 2010, 2011). The common thread here is that subordinates can increase the direct component of inclusive fitness by availing themselves of opportunities to lay eggs and raise brood, in their natal nest or elsewhere.

Facultatively social animals are ideal models for examining how resource competition influences the fitness balance between cooperation and competition, because they exemplify the costs and benefits of solitary vs. social living under varying ecological conditions (Schwarz et al., 2007; Wcislo and Fewell, 2017). Inter-individual competition should be most severe when critical resources are scarce, when population density is high, or both; competition for limited resources such as breeding sites should be particularly severe (Platt and Bever, 2009). In facultatively social bees, subordinate individuals with few opportunities for direct reproduction in their natal nests may be able to nest elsewhere, either by founding a new nest or joining a breeding group in which they will have more chances to lay eggs (Field et al., 2006).

The eastern carpenter bee, *Xylocopa virginica,* is a facultatively social species with a distribution that spans a large portion of eastern North America, from Florida in the south and southern Ontario in the north (Skandalis et al., 2011). They are floral generalists, but nest site specialists, and nests are costly to construct in terms of both time and energy. The value of the nest is demonstrated by the fact that frequently, they are occupied by successive generations of females, sometimes for decades (Rau, 1933). Eastern carpenter bees can nest solitarily or socially, and when social, females form small groups of two to five females, although there can be as many as eight (Gerling and Hermann, 1978; Vickruck and Richards, 2018). Females who are part of a social nest demonstrate one of three reproductive strategies (Richards, 2011). Dominant, primary females monopolize both foraging and reproduction and are first in a linear queue for reproductive opportunities, as only one female at a time is reproductive. Secondary females "wait" behind the dominant for opportunities to replace her, which can happen if she dies, becomes moribund, or occasionally, if the secondary aggressively usurps the dominant position (Vickruck and Richards, 2018). Finally, there are tertiary females, which are not a part of the reproductive queue and almost never leave the nest. These females do not reproduce even if primaries and secondaries are removed from the nest. By avoiding the physiological costs and risks of flight activity in their first year, they essentially double their life span and are able to successfully overwinter twice. They become primaries or secondaries in year two (Vickruck and Richards, 2018). From our experience, every social nest that was not newly constructed contains a tertiary female. Queue position is determined in early spring during a period referred to as the nestmate provisioning phase (NPP) when females often engage in aggressive in nest interactions as well as feeding each other (Richards and Course, 2015; Vickruck and Richards, 2017b). Females destined to be dominant and secondary females are often seen outside their natal nests at this time, briefly entering neighboring nests and presumably assessing whether it is best to remain in the reproductive queue in their natal nest or attempt to join a breeding group in another nest nearby. It is during this period that many females relocate to new nests (Peso and Richards, 2010). Thus, the breeding behavior of a facultatively social bee provides an ideal empirical model for evaluating the fitness consequences of cooperation and competition in shaping social behavior and group formation.

In this paper, we examine the behavioral consequences of high and low population density on *Xylocopa virginica* in two consecutive breeding seasons. We predicted that high population density would create more competition for nests and therefore more frequent social nesting and larger average colony size, as well as more competition for reproductive opportunities among females within breeding groups. We demonstrate that intense competition among females for nest sites increased both the frequency of cooperation (represented by nest-sharing) and the intensity of within-group competition for breeding opportunities. We genotyped females at 9 microsatellite loci to examine genetic relatedness among nestmates and used a randomization analysis to demonstrate that females were dispersing from their natal nests to ones that did not contain full siblings.

MATERIALS AND METHODS

Xylocopa virginica Colony Cycle

Eastern carpenter bees overwinter as adults in their natal nests, with females emerging from hibernation in spring (late April or early May in southern Ontario). The activity patterns of eastern carpenter bees are unusual for social bees, with two foraging flight periods (Richards and Course, 2015). The first phase is the nestmate provisioning phase (NPP) in which females bring back pollen to feed adult nestmates. During this time, many females make flights outside the nest, as whether they become the dominant or secondary is yet to be determined. The exception are tertiary females, who will not leave the nest unless they are the last remaining female, and if they do, never make a pollen trip (Vickruck and Richards, 2018). This behavior allows us to mark the vast majority of the females in an aggregation during the NPP. The second phase is the brood provisioning phase (BPP) in which the dominant female (also known as the principal forager) provisions brood cells on which only she lays eggs. If the dominant female dies, disappears or becomes moribund, the next female in line replaces her as the principal forager and egg-layer (Richards and Course, 2015; Vickruck and Richards, 2018). During NPP and as late as BPP, subordinate females often left their natal nests to join neighboring nests in the same aggregation, other aggregations, or in another location. Females were additionally categorized as resident or transient based on behavioral observations over the course of the foraging season. Resident females did not disperse and were only ever seen entering and exiting one nest during the BPP, while transient females dispersed from the nest in which they overwintered and were observed in more than one nest across the season. Transient females were only classified as such if they left their natal nest and permanently moved to a new nest. It was common for females to "shop around" for nests in the aggregation, sticking their heads inside multiple nests before deciding on a new nest, or ultimately returning to their home nest. Brood provisioning ceases in early July, after which adult females remain inside their nests with developing brood. When brood eclose at the end of the summer, they may be fed by older adult females that leave the nests during a brief, late summer foraging phase (late August or early September in southern Ontario) or leave to feed themselves before returning to their natal nest. The newly eclosed adults, both male and female, remain in their natal nests throughout the winter until the following spring.

In our study populations in southern Ontario, most females live 1 year, breeding in their first spring and summer following eclosion. The exception are the tertiary females, who do not forage in their first season, and are able to overwinter a second time. In more than 7 years of intensive behavioral observations, no female has ever been observed to forage in 2 consecutive years, so lifetime reproductive success is equivalent to the number of brood produced in one foraging season.

Description of Field Sites

We studied five nesting aggregations of *Xylocopa virginica*, each one located in a wooden bridge at the Glenridge Quarry Naturalization Site (GQNS), in St. Catharines, Ontario, Canada (43.122, -79.236 decimal degrees). This location is close to the northern edge of the species' range, with shorter flight seasons and much more severe winters than in southern locations where it has been previously studied (Gerling and Hermann, 1978). During the summers of 2012 and 2013, each bridge was home to between 10 and 22 nests. Bridges were constructed in 2003 and were available for the bees to use as nesting substrate beginning the spring of 2004. Eastern carpenter bees often reuse nests for many years. The term "new nest" refers to nests that were constructed in the current observation year, while "old nest" refers to nests that are being reused from previous years.

Bee Handling and Observations

In early spring, bees were trapped at their nest entrances using cup traps (Peso and Richards, 2011) on their first venture outside their natal nests. Cup traps are medium sized plastic cups that have a small hole cut out of the bottom while the top is covered with parafilm. The end with the small hole is then secured over the nest entrance with Velcro. Bees leave the nest but become trapped in the cup. Unmarked females were immediately placed on ice for approximately 10 min to allow for individual marking and measurements to be taken. Each female was individually marked on the thorax with a unique two-color combination using enamel model paint and her head width was measured across the widest part of her head, as a proxy for body size comparisons. The last tarsal segment of the left mesothoracic leg was removed and placed in chilled 100% redistilled ethanol for genotyping at a later date. Marked and measured bees were placed back outside of their nests to warm up and resume activity. Evidence from previous studies indicates that marking bees does not prevent them from relocating their nests (Richards and Course, 2015).

Observations of foraging females were used to determine which females were dominants (principal foragers) and which were subordinates. Foraging observations lasted from 08:00 h to 16:00 h during the NPP on days when there was no rain and the temperature was above 20°C. Time and nest of departure as well as the bee's individual paint ID were recorded for each bee leaving the nest. Bees were then released and the trap was replaced over the nest entrance. When a bee returned to the nest, the trap was removed to allow her entry. The time of her return, the nest to which she returned, as well as whether or not she was carrying pollen was recorded. At the end of the day all cup traps were removed for the night. Observations ceased for the season when an entire observation day passed (8 h) without seeing a single pollen trip by a female bee, indicating that the BPP was complete.

Assigning Nest Status

Nest status (social or solitary) was determined by observations across the entire season. Each time a female departed from the nest a small, flexible plastic transfer pipette was inserted in the nest entrance. If there was still a female present in the nest she would buzz, bite the pipette tip or block the entrance with her abdomen. The presence or absence of a guarding female was recorded and used to determine if the nest was social or solitary. Nests were classified as solitary if during the BPP only one female was ever seen bringing pollen to the nest and a second bee was never observed guarding the nest entrance. Nests were classified as social if more than one female was recorded in the nest during the BPP.

Genetic Analyses and Relatedness Calculations

DNA extraction and genotyping procedures are described in Vickruck (2014). In 2012, 189 females from 71 nests were genotyped. In 2013, 101 females from 64 nests were genotyped. Sixteen females were excluded from analyses of relationships in 2012 and 8 in 2013 due to missing data at more than 2 loci. To assess the relatedness among female nestmates during the winter, 19 nests were destructively opened in March of 2012 to reveal natal nestmates prior to emergence from hibernation and spring dispersal. Nests were opened by carefully planning away layers of wood to expose overwintering bees. All individuals inside nests were measured, marked and a tarsal sample was taken using the same techniques as used for summer bees. Because this procedure is destructive, it was only done for one time point so as not to destroy too many nests in our focal population.

Relatedness among female nestmates was calculated using the method described by Queller (1989) as implemented in the program Kingroup V2 (Konovalov et al., 2004). Kingroup V2 allowed us to differentiate which pairs of bees within nests were significantly more likely to be full sisters than unrelated pairs. Hymenoptera are haplodiploid (females are diploid while males are haploid) therefore full sisters inherit one of two maternal alleles and must inherit the single paternal allele. When comparing full sisters, this means that full sisters must share the paternal allele at all loci. We examined relatedness among nestmates at three successive time points: in late winter when females were still in their natal nests; in spring during the nestmate provisioning period, when females establish reproductive queues in social nests; and in summer during the

TABLE 1 | Female competition for nest sites varies with population density.

	Year		
	2012 High density	**2013 Low density**	**2012 vs. 2013**
Females marked	189	101	
Nesting location[a]			
Natal nest (residents)	74 (46%)	65 (64%)	X^2 = 8.70, d.f. = 2, P = 0.01
Different nest (transients)	34 (21%)	17 (17%)	
Disappeared	52 (33%)	19 (19%)	
Number of nests[b]	70	65	
Solitary	5 (7%)	30 (46%)	X^2 = 26.35, d.f. = 1, $P < 0.00001$
Social	65 (93%)	35 (54%)	
Colony size during the brood provisioning period (females per nest)	2.79 ± 1.03	1.86 ± 1.06	Mann Whitney U = 1167, $P < 0.0001$

Adult females were caught and marked when they first emerged from hibernation in the spring, so the number of females marked each year is an accurate measure of population density. Nesting location, number of occupied nests, and colony size were estimated during the brood provisioning phase in June and July, which lasted 31 days in 2012 and 32 days in 2013.

[a] *In 2012, 29 females in were caught flying through the nesting aggregation, so natal nest was unknown, and they could not be assigned resident or transient status.*

[b] *Newly constructed nests were included in the social nest category; 9 new nests, each of which contained 2 females, were constructed in 2012 when competition for nest sites was more severe, and no new nests were constructed in 2013.*

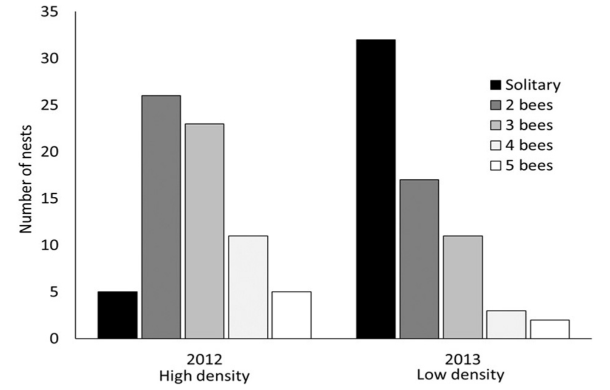

FIGURE 1 | Variation in group size during the brood provisioning phase of the colony cycle in response to high (2012) and low (2013) population density. In low density, significantly more females nested solitarily (**Table 1**, $P < 0.00001$, also see **Supplementary Table 1**). In both 2012 and 2013, the maximum colony size during the brood provisioning phase was 5 females.

brood provisioning period when dominant females provision their brood and lay eggs.

We created a randomization analysis to determine whether the observed numbers of sisters nesting together in each nest was different from the number of sisters that would be observed together if females were randomly distributed among nests. To do this we assigned all females marked in 2012 or 2013 to simulated nests at random. In each sample year, the number of nests as well as the size of each nest (the number of females recorded inside) was replicated exactly as observed in the sample population. After females were randomly assorted into nests, we used Kingroup V2 (Konovalov et al., 2004) to determine how many full sister pairs were present in simulated nests, as well as how many simulated nests contained full siblings. We then repeated this procedure 100 times for both the 2012 and 2013 datasets. Simulation results were used to create distributions for the expected number of siblings in nests and the number of nests that contained full siblings given the bees in the population for both 2012 and 2013. We then compared our observed values to the expected distributions of our randomization analysis to quantify the probability of our observations given the simulated data.

RESULTS

Evidence for Resource Competition Among Females

Since we caught and individually marked almost every individual bee that emerged from our study nests, population density was measured as the total number of bees marked each year (summarized in **Table 1**). In 2012 the population contained 189 females that occupied 70 nests (high density), while in 2013 it contained only 101 females in 65 nests (low density).

Under crowded, high density conditions, female bees should be more likely to share nests. As predicted, group size was strongly associated with population density (**Figure 1**). In high density conditions (2012), 96% of overwintered females nested socially (in groups) and average group size was significantly higher than in 2013 when population density was much lower and only 70% of females nested socially (**Table 1**). In 2012, females were also more likely to relocate from their natal nest, either to another nest in the population, to a location outside of the study area, or disappear from the population entirely (**Table 1**). Also in 2012, 9 females initiated new nests, a rate of new nest construction unprecedented at this location over 7 years of observations (2011–2013, 2016–2019); no new nests were initiated in 2013. In every instance of nest initiation observed at our study sites over about 7 years (several dozen examples), a single female excavated the nest entrance and the first sections of tunnel by herself. Thus, nest initiation is a solitary activity, although additional females frequently join the new nest within a day or two of nest entrance completion. The significant differences in group size between 2012 and 2013 demonstrate density dependence of social group formation, with strong competition for nest sites inducing higher rates of dispersal, higher rates of nest construction, higher rates of social group formation, and increased social group size.

Whereas group size was significantly higher in 2012 than in 2013, the duration of the BPP of the colony cycle was the same (**Table 1**). As a result, fewer subordinates would have achieved egg-layer status under high density, compared to low density.

Relatedness Among Nestmates

Relatively high relatedness among cohabitating females in winter suggests that many females could have formed kin groups

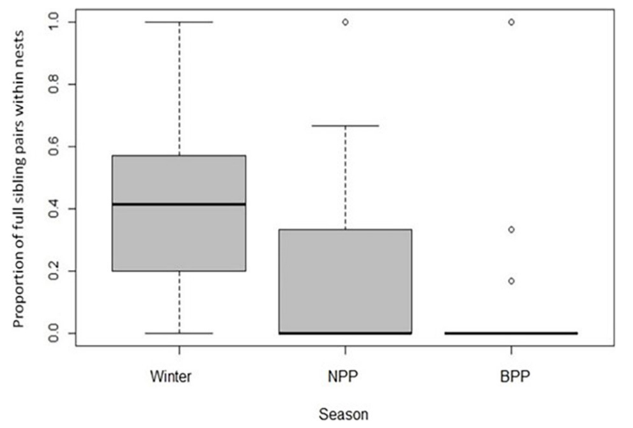

FIGURE 2 | Decline in kinship among social nestmates prior to the formation of breeding associations. Winter associations represent natal nestmates, since bees overwinter in their natal nests. The proportion of nest mate pairs that were full sisters decreased significantly from winter, through the spring nestmate provisioning phase (NPP) to the summer brood provisioning phase (BPP) (Kruskal-Wallis X^2 = 13.01, d.f. = 2, P = 0.001). Boxes-and-whiskers represent median and quartile ranges, while open circles represent outliers.

in spring by remaining together in their natal nests, yet the proportion of full sisters in colonies declined significantly from winter through spring to summer (**Figure 2** and **Table 2**), indicating that sibships were broken up as females dispersed to nests in spring. We therefore identified all possible full sisters for each genotyped female in the population and investigated whether they nested together or apart (**Figure 3**). Of 266 genotyped females that were still alive at the time of brood provisioning and egg-laying, only 30 sisters (11%) nested together, 178 sisters (67%) nested apart (in different nests), and 58 individuals (22%) did not have a full sister in the population (**Figure 3**). There was no effect between years (**Figure 3**). The proportion of sisters nesting together in summer was compared to the number that would be expected under a null hypothesis in which females were randomly distributed among nests (**Supplementary Figure 1**). The observed proportions of co-nesting sisters were not significantly different from random expectation.

DISCUSSION

Resource Competition Influences the Frequency of Social Nesting

In general, competition for resources is higher when population densities increase or when resources become scarcer (Moore et al., 2006; Platt and Bever, 2009). For large carpenter bees in general, and eastern carpenter bees in particular, nests are a critical breeding resource, costly to produce and in perpetually short supply (Ostwald et al., 2021a). Eastern carpenter bees forage on a wide variety of blossom types, but their nests are almost always found in structures built of milled lumber, especially pine and spruce (Hurd, 1978); thus they are foraging generalists but nesting substrate specialists (Vickruck and Richards, 2017a). They are strongly philopatric and nesting aggregations persist for years or even decades, as successive generations of females reuse nests (Rau, 1933; Gerling and Hermann, 1978; Richards and Course, 2015). Nests are very costly to construct; a female that initiates nest construction may take up to a week to construct a nest with a single tunnel, during a BPP that lasts only 3–6 weeks (note that nests are never founded jointly). As a result, most females attempt to breed in their natal nest or relocate to existing nests close by, usually in the same aggregation (Peso and Richards, 2011).

While population-level competition for available nest sites is the critical factor driving group formation in *X. virginica*, it also intensified within-group competition for breeding opportunities. When groups are larger, reproductive queues are longer, and subordinates in lower queue positions are less likely to achieve egg-layer status (Richards and Course, 2015). In our study population, subordinate females in queue positions 2 and 3 may eventually become principal foragers, as primary females (rank 1) often die before the end of the BPP (Richards and Course, 2015). However, females in ranks 4–6 never moved up to rank 1. Since dominant and secondary females virtually never forage in two consecutive breeding seasons (Vickruck and Richards, 2018), direct fitness for these two reproductive strategies is completely predicated upon raising brood in the current breeding season. Therefore, in a social nest of full sisters, individuals ranked lower in the queue would have no direct fitness. By relocating to a new nest and hopefully improving their ranks, a female would increase her direct fitness, while still maintaining the indirect fitness benefits of her egg laying sister in another nest. The significantly longer reproductive queues of 2012 would have led to significantly lower average direct fitness of subordinate females that year.

Unrelated Females Form Breeding Groups

Our results indicate that siblings overwintered together but rarely nested together. Kin relationships were broken up in spring as many females relocated to new nests. In low density conditions, such as those of 2013, females often were able to nest alone, either by relocating to a new nest or forcing their nestmates out, but in high density conditions, relocating females joined breeding groups of non-relatives. Most females take at least one flight during the NPP at the beginning of the season, which affords them the opportunity to assess the level of competition both within their home nest, as well as nests in the surrounding aggregation. This suggests that dispersal from the natal nest is a mechanism by which females solve the problem of severe competition for reproductive opportunities. This competitive cost is also mediated by the potential increase in direct fitness benefits from a female improving her position in the reproductive queue. A previous study demonstrated that transient females were as likely as resident females to become dominants in social nests, which means that transient females can successfully usurp primary queue positions (Richards and Course, 2015). The phenomenon of females joining nests of unrelated individuals,

TABLE 2 | Decline in mean relatedness among nestmates from the late winter hibernation phase to nestmate provisioning phase (NPP) to brood provisioning phase (BPP).

Colony phase	**Proportion of nests that contain full sisters**			**Within nest relatedness**		
	2012	**2013**	**Mean**	**2012**	**2013**	**Mean**
Winter	0.41 ± 0.29 (14)	NA	0.41 ± 0.29	0.35 ± 0.21 (14)	NA	0.35 ± 0.21
NPP	0.20 ± 0.33 (35)	0.30 ± 0.43 (24)	0.24 ± 0.38	0.24 ± 0.29 (35)	0.18 ± 0.40 (24)	0.21 ± 0.34
BPP	0.20 ± 0.38 (41)	0.17 ± 0.37 (20)	0.19 ± 0.37	0.19 ± 0.33 (41)	0.09 ± 0.42 (20)	0.16 ± 0.36
Relatedness across seasons	Kruskal-Wallis $X^2 = 13.01$, d.f. = 2, $P = 0.001$			2-way ordered ANOVA by season and year: $F_{(3,123)} = 1.70$, $P = 0.17$		

Values in brackets represent the number of nests. Queller-Goodnight estimates of average relatedness were calculated for all possible pairs of female nestmates within each colony, based on female genotypes at 9 microsatellite loci (Vickruck, 2014). The proportion of full sisters is the proportion of all possible female pairs in each nest whose genotypes suggested that they were full sisters, using Kingroup V2 (Konovalov et al., 2004).

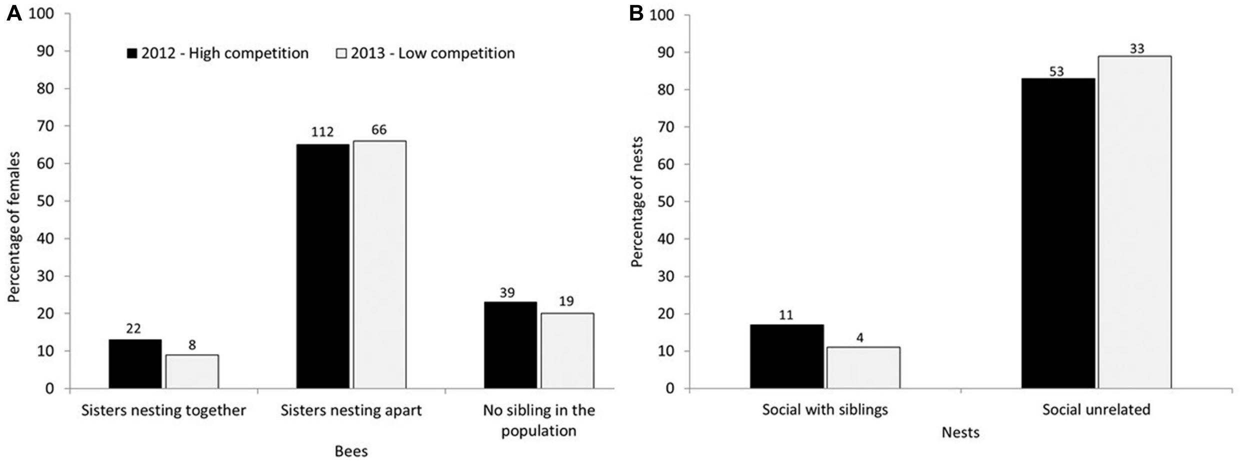

FIGURE 3 | Evidence for avoidance of competition by adult females during the brood provisioning period. **(A)** Although many females had at least one full sister in the population, most females nested apart from their sisters, rather than remaining together in the natal nest or dispersing together to a new nest. The proportions of sisters nesting together or apart did not differ between 2012 and 2013 ($X^2 = 1.38$, d.f. = 2, $P = 0.50$), suggesting that changes in population density did not influence the relatedness structure of colonies. **(B)** The proportion of social colonies that contained full sisters was also similar in 2012 and 2013 (Fisher's exact test, $P = 0.48$). Numbers above the bars represent the number of individuals **(A)** or nests **(B)**.

once thought to be an exception to the rule, is being noted more and more among social insects (Sumner et al., 2007; Peso and Richards, 2010; Leadbeater et al., 2011; Grinsted and Field, 2018). Indeed, relatedness among nestmates in *Xylocopa sonorina* is also highly variable, with many social nests comprised of unrelated individuals (Ostwald et al., 2021a, this issue). Recent modeling has demonstrated that species may be best to maximize high relatedness or low relatedness (social heterosis) to increase overall fitness (Nonacs, 2017). We would like to emphasize that we are not claiming that females are choosing new colonies randomly, but that their distribution among breeding groups appears to be random with respect to familial patterns.

While nesting with non-relatives means that transient females still incur the direct fitness costs of breeding competition within groups, it does allow them to reduce the indirect fitness costs of breeding competition with relatives. A female that manages to improve her queue position by moving to a new nest potentially increases her own direct fitness, but also avoids the cost of competing with siblings for reproductive opportunities. On average then, inclusive fitness should be higher for females that avoid sibling competition in their natal nests and move into nests occupied by non-relatives. Behavioral evidence demonstrates that females frequently investigate multiple new nests and groups before permanently relocating; even dominant breeders occasionally "visit" neighboring nests, sometimes spending several hours inside (Richards and Course, 2015). Another means by which females might avoid competing with siblings is by breeding in separate years. Tertiary females postpone reproduction until their second spring (Vickruck and Richards, 2018), perhaps avoiding competition with sisters that bred in their first year.

Since most social insects live in kin groups, most cooperative and helpful behaviors are directed at relatives. Eastern carpenter bees provide evidence of cooperation and helping behavior in non-kin groups, although the extent of their cooperative behavior is certainly more limited than in eusocial bees. Female carpenter bees recognize and are more tolerant toward nestmates and more aggressive to non-nestmates (Peso and Richards, 2011; Vickruck and Richards, 2017b). Both dominant and subordinate females have been observed to guard nest entrances against conspecific intruders. Perhaps the most dramatic example of cooperation occurs in early spring, prior to the onset of egg-laying and brood provisioning, when dominant females forage for pollen that they feed to subordinate nestmates, especially the lowest-ranking tertiary females that rarely leave the nest (Richards, 2011; Vickruck and Richards, 2017b). Such observations demonstrate that cooperative behavior does exist in insect societies in which most of the social group are unrelated.

Although this is a rare example of a structured insect society in which group members are mostly unrelated, there are intriguing hints in other social insects that non-kin-based sociality may often have been overlooked. To start with, many carpenter bees are facultatively social, nesting either solitarily or in small groups, sometimes as few as two females, in which only one female lays eggs, while other females await their turn to become the primary reproductive (Hogendoorn and Velthius, 1999; Ostwald et al., 2021b). Another carpenter bee, *Xylocopa sonorina*, also forms social groups comprised mostly of unrelated individuals (Ostwald et al., 2021a, this issue). Accumulating behavioral evidence suggests that some euglossine bees are facultatively social, forming social groups that resemble reproductive queues and which may include non-kin (Nascimento and Andrade-Silva, 2012). Recent phylogenetic evidence suggests that societies based on reproductive queues in which subordinates wait for their own reproductive opportunities could represent an early stage in evolutionary transitions to caste-based sociality, in which subordinates, sacrifice their own reproduction to aid kin (Schwarz et al., 2011; Richards, 2019). In recent years, considerable evidence has accumulated that subordinate individuals, variously known as "drifters," "aliens," or "joiners" leave their natal colonies and join unrelated colonies (Field et al., 2006; Brahma et al., 2019). Some wait for opportunities to inherit the role of dominant egg-layer (the "queen" role in eusocial colonies). Whether such joiners actively avoid moving to nests with relatives should be investigated. In many ants, unrelated gynes cooperate to initiate new nests, but once the first workers emerge, they aggressively and often violently, compete for dominance, with a single female becoming the colony's egg-laying queen (Haney and Fewell, 2018).

A rarely considered aspect of competition within social groups for resources critical to reproduction, is the effect of kin competition. Competition within breeding groups has different consequences for kin and non-kin. When individuals acquire disproportionately large shares of critical resources, they increase their own direct fitness at the expense of less competitive individuals. Competition among kin adds another layer of complexity: the increase in direct fitness experienced by dominant individuals may be obtained at the cost of lower direct fitness for related subordinates (Bach et al., 2006; Johnstone, 2008), so dominant individuals that harm kin achieve lower inclusive fitness than dominant individual that harm non-kin. Some theory predicts that the consequences of competition for resources within kin groups can be severe enough to "totally negate" the inclusive fitness benefits of kin cooperation (West et al., 2001; Bourke, 2011). The costs of kin competition can be avoided if related individuals disperse; indeed most empirical studies of kin competition and its effects have focused on how kin competition influences pre-breeding dispersal (Moore et al., 2006). However, dispersal has the additional effects of lowering opportunities for cooperative interactions that might increase access to breeding resources, as well as decreasing population viscosity and relatedness among potential cooperators. Therefore, dispersal in response to kin competition decreases the inclusive fitness costs of kin competition, but also decreases the inclusive fitness payoffs of cooperation and altruism (Bourke, 2011).

Eastern carpenter bees demonstrate alternative solutions to ways of coexisting in social groups when there is a division of labor. When the availability of nests is high, females can avoid competition by nesting solitarily, and when availability is low, they can nest in group while increasing their direct and indirect fitness benefits. Future research into the reproductive success of resident and transient females at different ranks in the reproductive queue across multiple years would help us to assign costs and benefits to each strategy under varying population densities. Further understanding patterns of paternity across the population will also be critical to assigning direct and indirect fitness benefits by reproductive strategy for this species.

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

JV and MR designed the experiment, performed statistical analysis, and wrote the manuscript. JV collected the data. Both authors have approved the final version of this 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.2021.738809/full#supplementary-material

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Cooperative Behaviors in Group-Living Spider Mites

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Cooperative behaviors are evolutionary stable if the direct and/or indirect fitness benefits exceed the costs of helping. Here we discuss cooperation and behaviors akin to cooperation in subsocial group-living species of two genera of herbivorous spider mites (Tetranychidae), i.e., the largely polyphagous *Tetranychus* spp. and the nest-building *Stigmaeopsis* spp., which are specialized on grasses, such as bamboo. These spider mites are distributed in patches on various spatial scales, that is, within and among leaves of individual host plants and among individual hosts of single or multiple plant species. Group-living of spider mites is brought about by plant-colonizing foundresses ovipositing at local feeding sites and natal site fidelity, and by multiple individuals aggregating in the same site in response to direct and/or indirect cues, many of which are associated with webbing. In the case of the former, emerging patches are often composed of genetically closely related individuals, while in the case of the latter, local patches may consist of kin of various degrees and/or non-kin and even heterospecific spider mites. We describe and discuss ultimate and proximate aspects of cooperation by spider mites in host plant colonization and exploitation, dispersal, anti-predator behavior, and nesting-associated behaviors and conclude with theoretical and practical considerations of future research on cooperation in these highly rewarding model animals.

Keywords: byproducts, direct fitness, indirect fitness, kin, kin selection, non-kin, spider mites, Tetranychidae

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BACKGROUND

Cooperative behaviors abound in animals but pose a challenge for evolutionary theory because of direct fitness costs to actors (helpers). Cooperative behaviors are likely to evolve whenever animals live together and interact for extended periods of time, and have been mainly examined in vertebrates and eusocial insects, and, here, especially among kin. In contrast, our insights into the occurrence and evolution of cooperation in non-eusocial group-living arthropods, and among non-kin and mixed kin and non-kin, are limited. Here, we give an account of various types of cooperative behaviors in group-living plant-inhabiting spider mites (**Figure 1**). These animals have been rarely subjected to research targeting cooperation but show various behaviors that clearly qualify as cooperation or suggest cooperation. We start with a theoretical delineation of cooperation, then introduce the biological and ecological features of spider mites that render them ideal animals to view certain behaviors from the perspective of cooperation and move on to report and discuss proximate and ultimate aspects of cooperation and cooperation-like behaviors of spider mites in selected behavioral-ecological contexts. The examples described are not meant to be exhaustive but to illustrate the diversity and ubiquity of cooperative behaviors in spider mites. We conclude our perspective article by highlighting the key features of cooperation in spider mites and point at opportunities and caveats in future research on this very topic and animals.

The Idea of Cooperation

Cooperation is defined as any behavior that has evolved, at least in part, to enhance the fitness of other individuals (e.g., West et al., 2007; Gardner et al., 2009). The fitness-enhancing (helping) individual is called the actor, and the fitness-enhanced (helped) individual is called the recipient. Actors incur some fitness cost, either directly because of temporary reduction of their individual fitness or indirectly because of increasing the fitness of others (fitness is a relative indicator; thus, if the fitness of recipients is enhanced, individual fitness of the actor is reduced, in relative comparison). As predicted by pertinent theories, such as kin selection (Hamilton, 1964) and reciprocity (Trivers, 1971), cooperation is only evolutionary stable if the actor is more than compensated for the costs of helping and obtains a direct fitness benefit *via* the enhancement of its individual fitness, and/or an indirect fitness benefit *via* the enhanced fitness of recipients sharing genes with the actor (Sachs et al., 2004; West et al., 2007; Gardner et al., 2009). These two mutually non-exclusive ultimate drivers of cooperative behavior may be proximately subdivided according to the mode of cooperation and route of fitness gain, with authors differing in the terminology of subtypes yet often having similar, strongly overlapping, or identical meanings. Direct fitness benefits, which are based on shared interests in cooperation, may arise from byproducts of otherwise selfish behaviors of the actor, and/or enforced cooperation, that is, rewarding cooperation and punishing non-cooperation (Gardner et al., 2009; originally dubbed reciprocal altruism by Trivers, 1971; similar to directed reciprocation *sensu* Sachs et al., 2004). Indirect fitness benefits may arise from population viscosity (i.e., limited dispersal passively leading to actors locally interacting more likely with kin than non-kin recipients; Hamilton, 1964) and/or kin discrimination (i.e., helping actors recognizing and preferentially interacting with kin recipients) and/or green beard effects (i.e., helping actors recognizing genetically pre-determined cooperation intents of helped recipients, no matter of their relatedness at other genetic loci) (e.g., Sachs et al., 2004; Gardner et al., 2009). Sachs et al. (2004) used the terms kin fidelity for site-specific helping kin, that is, in a given site, kin are more likely to encounter each other than non-kin, without the need of kin recognition (this also includes population viscosity), and kin choice for active kin discrimination. Further, these authors subdivided byproducts into one-way, which are behaviors that are not necessarily selected for cooperation, two-way, i.e., fitness-enhancing behaviors when performed in a group and include synergism (*sensu* Queller, 2011), and byproduct reciprocity. Queller (2011) extended inclusive fitness theory (Hamilton, 1964) to describe how cooperation may evolve between kin, kith (selection of neighbors who are neither kin nor kind *via* manipulation, actor-recipient choice, or actor-recipient fidelity feedback), and kind (based on green beard alleles).

Spider Mites as Cooperators

Here, we give an overview of cooperation and behaviors akin to cooperation by true spider mites (Tetranychidae), which clearly represent cooperative behaviors or which could qualify as cooperative behaviors yet we do not have enough information to judge whether these behaviors indeed qualify as cooperation according to the definitions described above. Spider mites (Tetranychidae) are globally distributed, mostly group-living herbivores (Helle and Sabelis, 1985 for review; **Figure 1**). Spider mites are highly rewarding model animals to view specific kin, non-kin, and heterospecific interactions from an evolutionary-grounded cooperation perspective, for a number of biological and ecological features. (i) As their name suggests, spider mites possess spinning glands in their mouthparts, with basic spinning types, such as *Tetranychus* spp., always and consistently spinning threads while walking (by every mobile life stage), and advanced types, such as some bamboo spider mites, being able to switch thread production on and off (Hazan et al., 1974; Saito, 1983; Clotuche et al., 2012). Over time, spinning threads result in three-dimensional webs on leaves and other plant parts, on, and under, which spider mites cohabit. The sophistication and complexity of jointly produced webs differ among spider mite genera and species, with the most advanced types being roof-like nests with protected entrances observed in grass spider mites *Stigmaeopsis* spp. (Saito, 1983). Accordingly, many cooperative behaviors of true spider mites and behaviors akin to cooperation are characterized and mediated by the joint use of webs produced by individual mites. Webs and single spinning threads are also beneficial as railroads and used for communication (Saito, 1983 for an overview). The ability to produce spinning threads is a decisive feature for the evolution of cooperative behaviors by spider mites. (ii) Most spider mite species are patchily distributed on their host plants and live in groups, with webbing being an important aspect of group formation and cohesion. (iii) The vast majority of spider mite species are arrhenotokous, that is, females produce haploid sons from unfertilized eggs and diploid daughters from fertilized eggs (Saito, 2010). Due to arrhenotoky, son-mother and, following mother-son mating, son/brother-sister coefficients of relatedness are 1, which has important implications to founder effects. (iv) Arrhenotoky allows colonizing host plants and the founding of patches/groups also by single immature or adult unfertilized females. While young mated females are the predominant dispersing life stage (Margolies and Kennedy, 1985; Li and Margolies, 1993; Azandémè-Hounmalon et al., 2014), also immatures, males and unfertilized females disperse (Brandenburg and Kennedy, 1982; Krainacker and Carey, 1990). For indirect fitness benefits selecting for cooperative behaviors of spider mites, the host plant colonization patterns seem as important as population viscosity in determining the kin structure within local patches and, in consequence, at regional levels. Due to arrhenotoky, any local patch founded by single females will, at least initially, result in high intra-group relatedness because of the possibility of mother-son and brother-sister mating. Patches founded by several females, or when other individuals later arrive on the host plant, may be composed of only kin if later arrivers come from the same source. Alternatively, they may represent mixed kin/non-kin

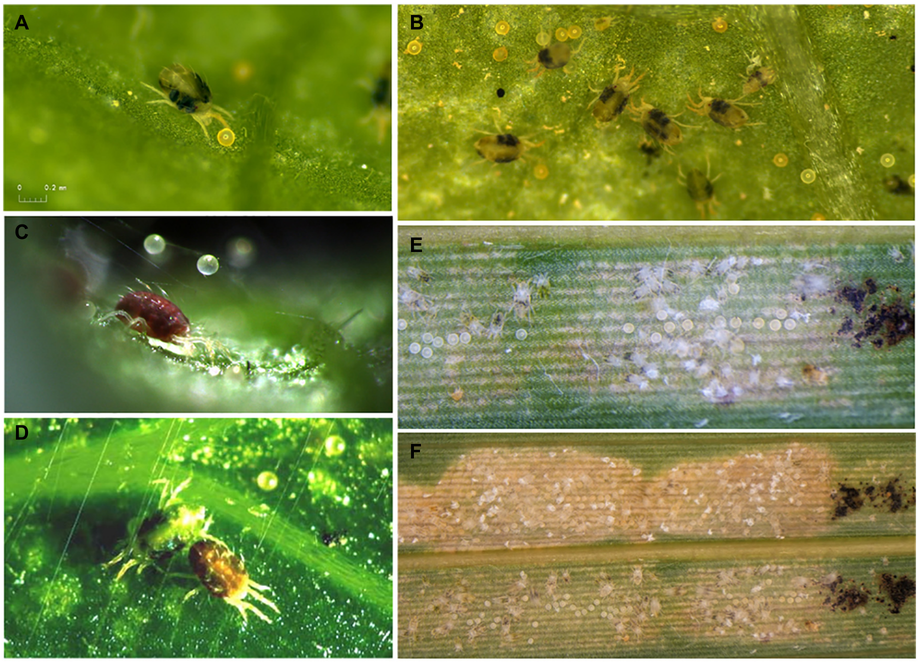

FIGURE 1 | (A) *Tetranychus urticae* female with egg, **(B)** group of *T. urticae* females and their offspring, **(C)** *Tetranychus kanzawai* female beneath spinning threads and eggs attached to threads, **(D)** *T. urticae* and *T. kanzawai* females sharing web, **(E,F)** nests of *Stigmaeopsis longus* on bamboo leaves, with all life stages and exuviae inside the nest and fecal piles outside the nest close to the entrance; the haze in **(E,F)** is caused by the woven roof of the nests; © **(A,B)** by PS, **(C,D)** by SY, and **(E,F)** by YS.

patches if the founders and later arrivers come from the same genetically heterogeneous source or if the founders and later arrivers come from genetically different sources. Nonetheless, even if simultaneous colonizers are genotypically heterogenous, kin individuals are more likely to interact with each other than with non-kin, at least until their density gets too high. The reason is that females deposit and aggregate their eggs at their feeding sites, which inevitably results in local kin subgroups within larger groups/colonies/patches. Whether it is single, several, or many individuals colonizing the same plant or site on a plant is tightly linked to the mode of dispersal and spinning thread-following behaviors, as described below.

Species and Behavioral-Ecological Contexts

We restrict our perspective of spider mite cooperation to species of two widely studied genera, that is, *Tetranychus* and the nest-building grass mite *Stigmaeopsis* (syn. *Schizotetranychus*), and consider interactions among kin, non-kin, conspecific, and heterospecific spider mites (**Figure 1**). The contexts looked at from a cooperation perspective comprise host plant colonization and exploitation, web-sharing for anti-predator benefits, and dispersal by *Tetranychus* spp., and nesting-associated behaviors by *Stigmaeopsis* spp. infesting bamboo and other grasses. Each behavioral-ecological context is illustrated by examples from the literature. We describe the current state of knowledge of behavioral characteristics and proximate aspects, and we contemplate and discuss whether the described behaviors have evolved for direct and/or indirect fitness benefits, which subtype of cooperation they seem to represent, and whether they require a given degree of genetic relatedness to enhance the fitness of both actors and recipients.

HOST PLANT COLONIZATION AND EXPLOITATION BY *TETRANYCHUS* SPP.

Depending on the mode of dispersal (Sabelis and Dicke, 1985 for an overview), i.e., whether by ambulation or by roping from exploited host plants or passively *via* the air and wind currents (either alone or collective; dubbed ballooning when mediated by the use of spinning threads, Bell et al., 2005), spider mites may colonize a new host plant either solitarily or as a collective. Collective ballooning is characterized by mites aggregating on the apex of leaves or plants, forming balls by joint webbing, and being carried away by the wind (e.g., Clotuche et al., 2011). Solitary ballooning has been reported in other spider mite genera (e.g., Fleschner et al., 1956), but in *Tetranychus* spp., ballooning usually represents collective behavior. Dispersal by roping and ambulation are basically and initially solitary behaviors, but if spinning threads are followed by others, they become collective.

On the host plants, spider mite females create local patches by ovipositing at feeding sites, but they also actively aggregate. Active aggregation may be proximately brought about by following the threads of other individuals (Yano, 2008; Astudillo Fernandez et al., 2012a), attraction to webbed areas (e.g., Clotuche et al., 2012, 2013a), and/or attraction to local changes in host plant chemistry (Kant et al., 2008; Rioja et al., 2017 for review). Also, visual and/or olfactory cues other than those present on the web, such as odor, shape, and/or color of other individuals or the host plant surface, may play a role. The ultimate reason why spider mites aggregate is that they benefit in fitness from other individuals and their webs and other microhabitat modifications in terms of egg production (Oku et al., 2009; Le Goff et al., 2010) and survival (Le Goff et al., 2010; Yano, 2012). Allee effects (Allee, 1931; Stephens et al., 1999) are a major driving force, i.e., benefits accrued by the presence of conspecifics in the immediate surrounding more than outweigh the costs of competition. Up to a threshold in abundance, individual fitness and group size are positively correlated. Accordingly, grouped spider mites commonly reach higher fitness than solitary spider mites if the environment (the accessible leaf area) is adjusted for exploitation competition (Le Goff et al., 2010). Positive group effects are byproducts of cooperation (or synergism *sensu* Queller, 1985) and provide direct fitness benefits regardless of intra-group genetic relatedness. Indirect fitness benefits play also a role in joint host plant exploitation and grouping because of founder effects, and females depositing and aggregating their eggs at local feeding sites, often result in patches where kin are more likely to interact with each other than with non-kin. Overall, these benefits commonly outweigh the costs of group-living such as intensified local and regional competition for shared resources, particularly food and mates.

Proximately, enhanced direct fitness by grouping may be brought about by reducing the intensity of individual web production (thinner, shorter, and/or fewer threads; Hazan et al., 1974) when others contribute to the shared web, and local (same leaf) and/or regional (systemic, on other leaves) favorable modification of plant biochemistry, such as breaking down the plant defense system resulting in more favorable nutritional quality of the shared host plant (Kant et al., 2008; Rioja et al., 2017), and/or more favorable leaf morphology (Oku and Yano, 2007). The energy saved in web production can be invested in reproduction (Le Goff et al., 2010). Joining existing webs and choosing between webs are not always necessarily in favor of kin-produced webs (Le Goff et al., 2012), which points at direct fitness benefits (byproduct cooperation) being the primary drivers of such behaviors. *Tetranychus* spp. has been suggested to possess kin discrimination abilities in activities such as spatial distribution (Le Goff et al., 2009), dispersal (Bitume et al., 2013), and mate choice (Schausberger and Sato, 2020). Experimental evidence suggests that *Tetranychus urticae* can also discriminate in site choice between group-labeled kin individuals from their own population (following inbreeding) and individuals from other populations and/or their products/environmental modifications and choose microhabitats that are most favorable, whether created by kin or not (Le Goff et al., 2012; Schausberger et al., 2019). When joining others on the same leaf or plant, later arrivers/followers, initially, recipients (either on the same leaf or other leaves of the same plant if systemic downregulation of plant defense has occurred) will benefit from pioneering colonizers who are initially the actors. Pioneers are later paid back by being released from costly individual web production and other aggregation-related benefits such as enhanced mating opportunities and enhanced protection from predators (see also the section on web sharing under predation risk), and other abiotic and biotic hazards for themselves and their offspring. Joining other groups and tightening the levels of aggregation enhance the chances of survival under predation risk because of attack abatement, even when the webbing is light or absent (Dittmann and Schausberger, 2017). An intriguing example of non-kin interactions comes from Schausberger et al. (2019), who showed that individuals of one population heavily benefited (without any direct interactions) from microhabitat manipulation by webbing or host plant biochemistry by pioneering colonizers from another population (Y coming to G environment), whereas in the reverse sequence, later arrivers were negatively affected (G coming to Y environment). This was possibly mediated by G- but not Y-individuals harboring endosymbiotic bacteria *Cardinium*, indicating that endosymbiotic bacteria may influence kin/non-kin cooperation in spider mites (Schausberger et al., 2019). In the sequence Y coming to G, pioneers may be paid back and benefit from later arrivers/followers because of a larger gene pool (G-Y mating is more favorable in terms of egg production than G-G mating). Therefore, in the sequence G coming to Y, Gs were initially actors and Ys were recipients; after arrival, Ys became actors by fertilizing Gs to the benefit of Gs (Ys paid costs because Y males prefer fertilizing G females).

Another possible benefit of joining others and aggregation may be broadening of local gene pools, allowing for the mixing of genotypes by mites following the spinning threads of, and joining, non-kin individuals. This would be considered an indirect genetic effect (IGE; Wolf et al., 1998; Santostefano et al., 2017) and may be beneficial, among others, because spider mites suffer from inbreeding depression (Vala et al., 2003; Le Goff et al., 2009; Yoshioka and Yano, 2014; Schausberger et al., 2019). IGEs could be one possible reason why females from a more prolific *T. urticae* line performed worse when mixed in a group with females from a less prolific line in the experiments by Le Goff et al. (2014). Whether and how pioneering colonizers of host plants and later arriving spider mites could benefit from IGEs affecting cooperation in exploiting host plants is little explored. Mites indirectly changing each other's behaviors *via* IGEs would be seen as byproduct cooperation but could also be a mix between direct byproduct benefits and indirect kin-selected benefits (Alemu et al., 2014).

Whether joining others is also beneficial for heterospecific spider mites may depend on the density and response of residents to later arrivers. For example, Sarmento et al. (2011) showed that *T. urticae* may benefit from the downregulation of plant defense by pioneering plant colonizers *Tetranychus evansi* (*T. evansi* helped *T. urticae* as a by-product because *T. urticae* produced more eggs when following *T. evansi*) but when the local abundance of *T. evansi* becomes too high, *T. urticae* is adversely affected by the dense webs produced by *T. evansi*, which may even lead to local exclusion and extinction of *T. urticae*. Possibly, such an interspecific interaction may be better dubbed facilitation, in addition to or instead of cooperation; facilitation between different herbivorous mite species has also been shown by Glas et al. (2014) and has been reviewed by Blaazer et al. (2018). The interspecific interaction reported by Sarmento et al. (2011) is not to be seen as cooperative if it is just *T. urticae* who benefits; however, it may be that *T. evansi* receives some other, hitherto unknown, initial benefit from the arrival/presence of *T. urticae*. Similarly, Godinho et al. (2016) showed for *T. evansi* and *T. ludeni* that plants previously infested with either con- or heterospecific individuals promoted later arrivers and boosted their fitness (higher egg production on previously infested plants) because of the downregulation of plant defense by pioneering individuals.

WEB SHARING AS AN ANTI-PREDATOR STRATEGY IN *TETRANYCHUS* SPP.

As outlined in the section on host plant colonization and exploitation, *Tetranychus* spp. females readily join webs established by others (Yano, 2008; Clotuche et al., 2013a) because of positive group (Allee) effects (Yano, 2008; Astudillo Fernandez et al., 2012a; Clotuche et al., 2013a). In addition, joining *Tetranychus* spp. females that would otherwise have to construct a new web alone gain direct web-mediated benefits of immediate protection against generalist predatory mites that have difficulties in coping with profuse spider mite webs (Yano, 2012). Such web-mediated protection confers considerable survival benefits because the vast majority of predatory mites are diet generalists (McMurtry et al., 1970; Sabelis and Bakker, 1992; Yano, 2012; Otsuki and Yano, 2014). Furthermore, the risk of predation of resident *Tetranychus* spp. females that built the webs are not increased by joiners, and at low densities, there are no negative host plant-mediated group effects (Yano, 2012). This indicates that the direct costs of sharing fresh webs are low or negligible. Altogether, considerable direct fitness benefits and low costs to resident females promote web sharing under predation risk, independent of kinship, and, thus, represent byproduct cooperation. This explanation is supported by the fact that heterospecific *Tetranychus* spp. females, such as *T. urticae*, *Tetranychus kanzawai*, and *T. evansi*, may share webs under predation risk in a similar way as conspecifics do (Yano, 2012; Sato et al., 2016). *Tetranychus* spp. females usually oviposit on the surface of leaf undersides. However, when they are threatened by specialist predatory mites that are well able to cope with webs (Sabelis and Bakker, 1992) and feed preferentially on spider mite eggs (Blackwood et al., 2001; Furuichi et al., 2005a), they disperse from invaded patches (Bernstein, 1984; Grostal and Dicke, 1999; Fernández-Ferrari and Schausberger, 2013; Hackl and Schausberger, 2014; Freinschlag and Schausberger, 2016; Otsuki and Yano, 2019) or oviposit on the webs instead (Oku and Yano, 2007; Lemos et al., 2010; Murase et al., 2017). For example, in environments with *T. kanzawai* eggs on and off the web, the predatory mite *Neoseiulus womersleyi* largely refrains from killing eggs on webs. Shifting oviposition toward webs is a type of maternal care that reduces offspring predation risk and thereby confers direct fitness benefits (Otsuki and Yano, 2017). Other ovipositing females benefit from existing webs under predation risk (byproduct cooperation). Under no or low predation risk, *Tetranychus* spp. females usually do not deposit eggs on webs away from the leaf surface, which points at fitness costs of oviposition on webs. Costs may include delayed and/or more complicated access to the leaf surface by hatching offspring and/or eggs on webs away from the leaf surface being more strongly exposed to abiotic hazards such as rain and wind (Okada and Yano, 2021).

COLLECTIVE DISPERSAL BY *TETRANYCHUS* SPP.

Collective Ambulatory Dispersal by Following Spinning Threads

Tetranychus spp. females disperse on and between leaves of their host plant primarily by walking (Brandenburg and Kennedy, 1982; Margolies and Kennedy, 1985). Ambulatory dispersing *Tetranychus* females often follow spinning threads, functioning as trails, left by preceding females. Follower females reinforce the trails with their own spinning threads, providing an opportunity for collective choice of dispersal direction (Yano, 2008). Although ambulatorily dispersing *Tetranychus* females do not consistently display collective choices of feeding and oviposition sites (Astudillo Fernandez et al., 2012b), collectively dispersing *Tetranychus* females may gain byproduct benefits from sharing webs at the new feeding site, while *Tetranychus* females not following trails become solitary founders of new colonies with initially high intra-colony relatedness (Yano, 2008). Local colonies founded by solitary females may later merge into extended high-density patches representing an ensemble of local kin patches. The reasons why such collective choices do not always occur are debated (Astudillo Fernandez et al., 2012b). Collective site choices in environments with specialist predatory mites that use spider mite threads for prey-searching are costly (Roda et al., 2001; Furuichi et al., 2005b; Shinmen et al., 2010). Therefore, whether collective dispersal is more advantageous than solitary dispersal is thought to depend on the strength of "positive group effects" in new habitats (Astudillo Fernandez et al., 2012a). Whether spinning thread-following behavior is influenced by genetic relatedness between pioneers and followers is unclear, but it is often kin individuals that disperse from the same patch. Bitume et al. (2013) showed that both increased local density and closer genetic relatedness increased the ambulatory dispersal distance of *T. urticae*. Since direct fitness benefits accrue anyway, indirect benefits arising from local kin neighborhoods may be considered jointly acting or secondary selective forces of thread-following behavior.

Collective Aerial Dispersal by Ballooning

Besides ambulatory dispersal, *Tetranychus* females also disperse aerially, either alone (Smitley and Kennedy, 1985; Margolies, 1987) or as part of a woven ball (dubbed ballooning; Bell et al., 2005), which may contain both adults and immatures. Ballooning mites can also be phoretic if the balls are carried away by other animals (Brandenburg and Kennedy, 1982;

Clotuche et al., 2011, 2013b). For collective ballooning, mites start to move to the apex of leaves and plants, and others follow the spinning threads to jointly produce webbing and form balls on the apex. Depending on the delay between the initiation of ball formation and take-off and the size of the balls, all ballooning mites survive and are carried away, or early arrivers are trapped inside and die and only those joining the ball at a later time survive until being carried away by the wind. Collective dispersal *via* ballooning could represent cooperation based on the expression and recognition of green beard alleles that may indicate kinship or not. Clotuche et al. (2013b) observed that *Tetranychus* individuals did not discriminate and segregate with kin during ball formation; however, this may have been due to mixed rearing before the experiment, allowing familiarization among kin and non-kin. Also, these experiments do not rule out a possible role of kin selection, because on a local scale *Tetranychus* individuals live more likely with kin than non-kin and, thus, may not need to discriminate who initiated or joins in ball formation. If usually formed by kin, individuals initiating ball formation could be considered kin-selected true altruists (indirect fitness gain outweighing direct fitness loss; Kay et al., 2019) because those individuals (actors) may be enclosed and die inside the balls, but may gain indirect benefits by helping kin recipients to disperse (Clotuche et al., 2011). Whether mites dying inside balls sacrifice themselves to aid in ball formation or are trapped accidentally by other mites requires close scrutiny. In any case, dying inside the balls just occurs if there is a long delay between initiating ball formation and being carried away by the wind; if the take-off occurs soon after initiation of ball formation, there are no dead individuals inside the balls (Clotuche et al., 2013b). One likely selective force of collective ballooning may be immediately acting Allee effects on the new host plant (byproduct cooperation), i.e., collective colonization of a new host plant increasing individual fitness because of positive group effects (synergism *sensu* Queller, 1985) as compared to solitary colonization (Clotuche et al., 2013a,b). Cooperation in forming high density aggregations on tips of overexploited host plants may also counter dehydration (byproducts), as has been shown for the house dust mite *Dermatophagoides farinae* (Glass et al., 1997).

COOPERATIVE BEHAVIORS OF GRASS SPIDER MITES, *STIGMAEOPSIS* SPP.

Eusociality has not yet been observed in mites, but cooperative brood care and overlapping generations, which correspond to the intermediate subsociality II degree of sociality (following the "nest building" subsocial route to eusociality; Michener, 1969; Wilson, 1971), are present in group-living spider mites (Saito, 2010). While *Tetranychus* spp. are also considered subsocial, some *Stigmaeopsis* spp. show advanced social organization (Saito, 2010). The genus *Stigmaeopsis* comprises a number of species that infest leaves of bamboo and other grasses in Asia; some species have been unintentionally introduced and are now established in the Americas and Europe by the bamboo trade (Ostoja-Starzewski, 2000; Pratt and Croft, 2000; Kiss et al., 2017). A remarkable feature of *Stigmaeopsis* spp. is the construction of tunnel-like nests by spinning threads along the veins and edges on the lower surface of leaves. The mites feed, develop, and reproduce inside the nests. Until two decades ago, the genus *Stigmaeopsis* was regarded as a single species, *Schizotetranychus* (syn. *Stigmaeopsis*) *celarius* Banks. However, recent studies found differences in the range of host plant species, nest and group sizes, and in cooperative behaviors, such as nest building, enlarging, and repairing, waste management, nest defense against predators, and male-male aggression, resulting in the description of 15 species (Saito et al., 2004, 2018, 2019). In the following, we highlight four aspects of cooperative behaviors in the genus *Stigmaeopsis*, that is, nest building, nest/brood defense, male-male aggression including fights for females, and social immunity.

Cooperative Nest Building and Nest/Brood Defense

Predation pressure is a strong selective force for the evolution of sociality (e.g., Wilson, 1971). Woven nests of *Stigmaeopsis* spp. provide some physical protection from predators. However, several predators, such as the predatory mite *Typhlodromus bambusae*, are able to intrude into the nests, especially through nest entrances. *Stigmaeopsis* spp. (*Stigmaeopsis miscanthi*, *Stigmaeopsis sabelisi*, *Stigmaeopsis longus*, *Stigmaeopsis celarius*, and *Stigmaeopsis nanjingensis*) that construct extended large nests show cooperative brood defense (counterattack) by males against intruders. Nests of *S. miscanthi* are sometimes only occupied by a single male and then the male and females jointly defend the nest (Saito, 1990). Nests of *S. longus* (**Figure 1**) are commonly inhabited by several males, which jointly defend the nests. Adult mites (biparental, i.e., both males and females, or just males) drive potential intruders away from nests by pursuit, jabbing, and beating, and, sometimes, even kill immature predators (Saito, 1986a,b; Yano et al., 2011; Saito and Zhang, 2017). The success rate of counterattack varies among species (Mori and Saito, 2005), and increases as the number of adult mites in a nest increases, i.e., the success of counter-attacks positively correlates with the size of nests (Saito, 1986a,b; Yano et al., 2011; Saito and Zhang, 2017). Such cooperative defense behaviors seem absent or less effective in species that construct separate small nests, such as *S. takahashii* and *S. saharai* (Mori and Saito, 2005). However, their nests are so small and the nest entrances are so narrow that predators are rarely able to intrude. As a consequence, the physical protection provided by the small nests is much higher than that of extended large nests (Mori and Saito, 2004). Moreover, separate scattered nests decrease the success of predators in detecting nests with live prey inside, because nests with sucked-out prey corpses can function as a trap for predators (Saito et al., 2008). Altogether, these studies suggest that differences in nest and group sizes in the genus *Stigmaeopsis* are associated with divergence in anti-predator strategies: cooperative defense by counterattacking predators in large groups and constructing smaller more protective nests in small groups. Counterattacks against potential intruders protect their own and the offspring of nestmates but incur the costs of being killed by predators. Therefore, nest size and cooperative defense are regarded as key traits in the evolution of grass spider mite sociality.

Male-Male Aggression and Fight for Females

The group of *S. miscanthi* species (*S. miscanthi* HG and ML forms, *S. sabelisi*, *S. continentalis*, and *S. formosa*) infests *Miscanthus* spp. grass, enlarges and extends their nests over time, and counterattacks predatory intruders (Saito et al., 2018, 2019; Sato et al., 2019). Adult males are not only aggressive against predators but also against conspecific males and may even kill each other to establish a harem (Saito, 1990). *Stigmaeopsis longus* (**Figure 1**) engage in precopulatory mate guarding without lethal fighting, whereas *S. miscanthi* males may fight to death inside nests when competing for females. However, the intensity of male-male aggression, quantified by the frequency of lethal male fights, varies among species and among populations in the *S. miscanthi* species group and seems to correlate with winter harshness (Saito, 1995; Saito and Sahara, 1999; Sato et al., 2013). Winter harshness can mediate average genetic relatedness among nestmates in the *S. miscanthi* species group because mother-son mating during spring colony-establishment occurs more likely in colder than warmer regions (Saito, 1995; Sato et al., 2013). Therefore, kin selection is a plausible explanation of the geographic variation in male-male aggression. Non-lethal fighting may represent cooperation by non-killing actors helping kin recipients to survive and reproduce at the expense of a decrease in the direct fitness of the actor but an increase in indirect fitness (Saito, 1995; Saito and Mori, 2005). Alternatively, lethal fighting could represent spite (Hamilton, 1970; Foster et al., 2001; Gardner and West, 2006; Sato et al., 2013).

Social Immunity: Cooperative Nest Sanitation and Waste Management

Social immunity is defined as "any collective and personal mechanism that has emerged and/or is maintained at least partly due to the anti-parasite defense it provides to other group members" (Meunier, 2015). In nest-building organisms, social immunity is achieved by nest sanitation behaviors to prevent or reduce disease transmission and keep the living space inside nests clean. Waste management is widespread from communal to eusocial species (Jackson and Hart, 2009) and is closely associated with the evolution of sociality in the Acari (Saito, 1997). Some species of the genus *Stigmaeopsis* show obvious waste management (**Figure 1**). For example, the *S. miscanthi* HG form, which lives on *Miscanthus* spp. grass, constructs large woven nests by continuously extending its nests. In exceptional cases, large nests may be inhabited by more than a hundred individuals with three overlapping generations (Saito et al., 2000). Inside the nests, one or several fecal piles, spaced at similar distances, may be found. Fecal piles emerge by two simple behavioral rules: mites deposit their feces at sites with previous feces; in absence of previous feces, they deposit their feces inside the nest close to one of the two entrances (Sato et al., 2003). The mites recognize fecal sites by volatile chemical cues and the nest entrance by tactile cues (Sato et al., 2003). Similar waste management has been observed in *S. longus*, which also constructs continuously enlarged nests but infests bamboo plants. However, in this species, the first fecal pile is deposited outside nests (Sato and Saito, 2006; **Figure 1**). *Stigmaeopsis takahashii* and *S. saharai*, which also infest bamboo plants but rather construct separate new nests than expand existing nests, deposit their feces outside the nest entrances, and do not respond to volatile chemical cues (Sato and Saito, 2006, 2008). Therefore, the use of volatile chemical cues in waste management is thought to have co-evolved with extending and enlarging existing nests. In *S. longus*, additional highly sophisticated nest cleaning behaviors have been reported. Females keep spinning threads after nest construction, which not only function to reinforce the nests but also to remove exuviae and other dust, possibly containing pathogens, scattered on the leaf surface inside nests (Kanazawa et al., 2011). To this end, females walk in a zigzag pattern and spin threads that are soft and sticky when fresh. These threads trap the exuviae and dust from the floor (the leaf surface) of the nests. Females push the trapped exuviae and dust up and glue them to the woven roof of the nest, resulting in a clean leaf surface inside the nests beneath the roof. Cooperation in nest building and social immunity activities have clear direct benefits, so arise from byproducts, but it is more than plausible to also assume a role of kin selection in these behaviors and indirect fitness benefits since it is usually and predominantly kin that live together and enlarge nests (kin fidelity *sensu* Sachs et al., 2004).

CONCLUSION

For most behavioral contexts looked at, cooperation by spider mites is based on shared interests between partners, that is, byproduct cooperation. In interactions such as host plant exploitation, collective dispersal, and shared nests, closer than average genetic relatedness is a likely consequence of host plant colonization and settling processes inevitably resulting in more frequent and more likely encounters between kin than non-kin. Thus, partners may additionally benefit from close genetic relatedness to additionally obtain indirect fitness gains. Whether these cooperative behaviors have evolved because of close kinship or are more likely to occur among kin than non-kin is a readily testable hypothesis if requiring kin discrimination, but is more difficult to test if they are due to founder effects and/or population viscosity (here, individuals do not have to actively recognize kin to more likely interact with kin than non-kin). However, because *Tetranychus* spider mites commonly live in high-density patches, it is very unlikely that they evolved fine-scale kin discrimination abilities, such as among siblings, aunts, and nieces, but group-level discrimination abilities, such as among populations, subpopulations, and lines, are obviously present (Le Goff et al., 2009, 2014; Schausberger et al., 2019; Schausberger and Sato, 2020).

Highly important aspects to consider in future studies that address the question of whether cooperative behaviors of spider mites evolved for direct and/or indirect fitness benefits are the origin, sampling, and rearing history, and with that the level of relatedness and familiarity, of the individuals used for experimentation. Considering the high intrinsic rates of increase of spider mites and patchy distribution, laboratory populations founded by specimens sampled in the wild from only one site or plant may present little genetic variation. Moreover, spider mites commonly have long been reared in the laboratory before being subjected to experiments and may, thus, represent inbred (sub)-populations or lines with close average inter-individual relatedness. Also, joint vs. separate rearing is an issue if group-level kin recognition is brought about by shared local or regional features of the environment (such as a shared host plant) serving for environmentally acquired population or line-specific labels. Thus, studies are prone to fail in establishing sufficient genetic or environmentally acquired variation between kin and non-kin or among differing degrees of kinship if just using individuals of one and the same inbred population for cooperation, kin recognition, and other topically pertinent studies.

Overall, our brief reports of selected behavioral-ecological contexts, considerations, thoughts, and views of cooperation and behaviors akin to cooperation in spider mites emphasize the great potential and experimental suitability of these animals for addressing fundamental questions in the cooperation framework. This perspective article may serve as a base and starting point to stimulate, guide, and/or intensify research on this exciting topic using spider mites as highly rewarding model animals.

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

PS conceived the study idea and coordinated the writing. PS, SY, and YS wrote the original draft of the manuscript and contributed to manuscript revisions. All authors contributed to the article and approved the submitted version.

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Fluid Nest Membership Drives Variable Relatedness in Groups of a Facultatively Social Bee

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Kin selection theory has dominated our understanding of the evolution of group living. However, many animal groups form among non-relatives, which gain no indirect fitness benefits from cooperating with nestmates. In this study, we characterized the relatedness and inter-nest migration behavior of the facultatively social carpenter bee, *Xylocopa sonorina*. Nesting constraints due to costly nest construction in this species give rise to intense intraspecific competition over access to existing nests. We used mark-recapture techniques to characterize patterns of dispersal and nest relocation within a nesting aggregation of spatially clustered nests. Two-thirds of bees relocated at least once during the reproductive season, likely to seek reproductive opportunities in another nest. This fluid nest membership creates opportunities for association among non-relatives. To assess the effects of this dynamic nesting behavior on group relatedness, we used microsatellite analysis to estimate relative relatedness within and between nests in the aggregation. We found that relatedness was variable across sampling years, but that in many cases nestmates were no more related to one another than they were to non-nestmate bees in the population. Together, these results suggest that group composition in *X. sonorina* may result from strategies to maximize direct fitness. This study supports the hypothesis that factors beyond kinship, such as ecological constraints, are likely to drive group formation in this species.

Keywords: carpenter bees, *Xylocopa*, microsatellite, dispersal, social evolution, non-kin, relocation, drifting

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INTRODUCTION

For many animal groups, kin selection theory has served as the central paradigm for understanding the evolution of social behavior (Hamilton, 1964; West-Eberhard, 1975; Trivers and Hare, 1976). Nevertheless, many animals form social groups with non-kin, and gain little to no indirect fitness benefits from cooperation (Bernasconi and Strassmann, 1999; Clutton-Brock, 2009; Riehl, 2013). Particularly within the Hymenoptera, these groups tend to be understudied relative to kin groups, but offer valuable opportunities to test hypotheses about drivers of social evolution while controlling for indirect fitness benefits (Ostwald et al., in review, this issue). However, the extent to which animals form alliances with non-relatives and the mechanisms by which these groups arise remain unknown for many social taxa.

Non-kin groups may arise through shared exploitation of limiting resources, especially nesting sites. These conditions may prompt individuals to disperse and seek reproductive opportunities by joining established groups or constructed nests. Nest joining by non-relatives is common within the cooperatively breeding birds, which may gain direct fitness benefits of cooperation even when relatedness is low (Piper et al., 1995; Young, 1998; Baglione et al., 2002; Riehl, 2011). Likewise, among the communal and polygynous wasps and bees, females may join nests established by non-relatives, where they may benefit from reduced costs of guarding, provisioning, and/or nest construction (Danforth et al., 1996; Johnson, 2004; Wcislo and Tierney, 2009; Mora-Kepfer, 2014; Ostwald et al., in review, this issue).

The large carpenter bees (genus *Xylocopa*) represent useful candidates for testing hypotheses about social evolution, particularly in the context of non-kin sociality. Carpenter bees may be solitary or may form small, fluid societies in which a single dominant female performs all or most of the egg laying, provisioning, and nest construction/maintenance (Gerling et al., 1989; Richards and Course, 2015; Buchmann and Minckley, 2019). Subordinate females may perform guarding duties but otherwise contribute little to the productivity of the nest (Hogendoorn and Velthuis, 1993; Richards, 2011; Prager, 2014). Instead, subordinates are likely waiting for opportunities to inherit existing nests (Velthuis and Gerling, 1983; Richards, 2011; Schwarz et al., 2011; Vickruck and Richards, 2018), which can be less costly than new-nest construction (Ostwald et al., 2021).

Because nests are costly and valuable resources, most females will breed in existing nests rather than undertaking new nest construction (Peso and Richards, 2011), which is energetically expensive (Ostwald et al., 2021). This limitation creates a shortage of available breeding space that can give rise to intense intraspecific competition for reproductive opportunities (Gerling et al., 1989; Buchmann and Minckley, 2019). Following emergence, adult *Xylocopa* often (but not always—see Gerling, 1982; Velthuis, 1987) overwinter with siblings in the natal nest in mutually tolerant pre-reproductive assemblages that become aggressive and break up at the onset of the reproductive season (Michener, 1990). These family groups become aggressive at the onset of the reproductive season, prompting dispersal and the formation of dominance hierarchies (Velthuis, 1987; Michener, 1990; Richards and Course, 2015). To secure reproductive opportunities, females may compete for dominance in their natal nests or may attempt to usurp reproductives in nearby nests (Hogendoorn and Leys, 1993; Hogendoorn, 1996; Richards, 2011). Alternatively, females may disperse from their natal nests to join neighboring nests, perhaps seeking to advance their position in a reproductive queue or to minimize competition with close kin (Vickruck and Richards, 2018, 2021).

Nest joining behavior has been observed in several *Xylocopa* species and is expected to create opportunities for association among non-relatives (Gerling, 1982; Velthuis, 1987; Camilo and Garofalo, 1989; Peso and Richards, 2011). Peso and Richards (2010) used mark-recapture techniques to examine the extent of nest joining in the eastern carpenter bee, *Xylocopa virginica*, and found that roughly half of recaptured females were found at a different nest from the one at which they were originally marked. The high rate of relocation can explain low within group relatedness in social groups of this species (Vickruck and Richards, 2021). Aside from this study, genetic relatedness of nesting groups is unknown for any other species of carpenter bee, despite ample behavioral observations indicating that carpenter bees tolerate non-relatives in their nests.

We examined nest joining behavior and relatedness in the facultatively social valley carpenter bee, *Xylocopa sonorina*. Like most carpenter bees, this species is characterized by high reproductive skew and intense nest-site competition (Gerling, 1982). Gerling (1982) observed adult females joining active nests during the reproductive season, and also found that some recently emerged offspring dispersed from their natal nests soon after emergence. We predicted that dispersal and nest relocation may dilute relatedness within nests, leading to mixed associations of kin and non-kin. Using complementary behavioral and genetic approaches, we characterized the dynamic group membership of *X. sonorina* and the consequences of these behaviors for relatedness within and among nesting groups. In doing so, we aim to highlight mechanisms of group formation that can evolve in the absence of helping behavior and indirect fitness returns.

MATERIALS AND METHODS

Study Design and *X. sonorina* Seasonal Activity

To characterize nest relocation and relatedness patterns in *X. sonorina*, we collected genetic and behavioral data from a single nesting aggregation (an occupied log of Goodding's willow, *Salix goodingii*, 206 cm length × 23 cm diameter) sourced from a riparian area in Phoenix, AZ, United States (33.41988 N, −112.07062 W). In central/southern Arizona, winter quiescence for *X. sonorina* typically ceases in March (Minckley, 1987). Mating activity occurs in March and April (Minckley and Buchmann, 1990), and female reproductive activities, including nest construction/renovation, egg laying, and offspring provisioning, occur primarily in April and May (Minckley, 1987; Ostwald et al., 2020), and offspring emerge in late May to June (Minckley, 1987; Ostwald et al., 2020). This species is univoltine and produces an average of 11.5 brood per nest (Ostwald et al., 2020), laid by a single reproductive female. Nests may be solitary or may contain as many as 9 adults during the spring (Ostwald et al., 2020), though the distribution of group sizes is expected to depend strongly on local factors such as nesting density.

To capture dynamic nest movement behavior across the reproductive season but prior to offspring emergence, we conducted behavioral observations from mid-March to early May of 2021. Likewise, to capture group relatedness at the onset of dispersal and reproductive activity we collected genetic samples in late March to early April of 2019 and 2020.

Behavioral Observations of Dispersal and Nest Joining

We used mark-recapture techniques to examine dispersal and nest joining by female bees over the course of the reproductive season. During spring 2021, we caught and/or observed bees entering and departing nest entrances in our focal log (as in Peso and Richards, 2010; Peso and Richards, 2011). Upon first capture of an individual, we recorded the nest of origin and marked each bee with a unique two-color paint marking on the thorax and abdomen using Testors enamel paint (Testors, Vernon Hills, IL). For all subsequent observations we recorded the identity of the bee and the nest of departure or arrival. These nests may have been natal nests or non-natal nests to which they had dispersed. To estimate total population size, we extrapolated from counts of the number of marked and unmarked female bees entering and exiting the log over the course of 1 h at the end of the spring, after all focal bees had been marked.

We observed nest entry and departure activity for 17 days between March 18 and May 2, 2021. On each sampling day we observed bees for 1–4 h within the daily window of peak flight activity, for a total observation period of 30 h 25 min over the course of the spring. We observed all entries and exits during these observation periods, and recorded the nests visited. Ambient temperatures at the time of observation ranged from approximately 20–34°C.

Genetic Analysis

We collected genetic samples by capturing bees upon departure from their nests. We anesthetized all females on ice then removed the most distal tarsal segment from one metathoracic leg using a sterile razor blade. Removal of this tarsal segment is not known or expected to significantly impair mobility (Vickruck and Richards, 2017). Tarsal samples were stored in ethanol at −20°C for later genetic analysis.

We extracted DNA from all tarsal samples using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, United States). We amplified DNA at 6 microsatellite regions characterized for the congener *X. virginica* (Vickruck, 2015: XV7, XV9, XV27, XV28, XV30, XV42), having previously confirmed the presence of these loci in *X. sonorina* using gel electrophoresis of amplified PCR products. Loci were amplified in three 12.5 μl PCR multiplex reactions of two or three primers per multiplex. Forward primers were tagged with a fluorescent probe (6FAM, TET, PET, HEX, or VIC) for fragment identification (**Supplementary Table 1**). Genotypes were analyzed by fragment analysis and scored by visual inspection of the tracefiles using Geneious R8 (Kearse et al., 2012).

Relatedness Calculation and Statistical Analysis

We estimated relative relatedness of sampled individuals using methods developed by Queller and Goodnight (1989), using the R package *related* (Pew et al., 2015). Data are presented as pairwise comparisons of the relative relatedness of all possible pairs of individual female bees in the sample. We used Wilcoxon tests to compare relative relatedness of nestmates (within nest comparison) vs. non-nestmates (between nest comparison) within each year of collection. We excluded from analysis any individuals that were missing genotype information at two or more loci (N = 6). In addition, we tested for adherence to Hardy-Weinberg equilibrium at all loci, and estimated F_{ST} and F_{IS} between collection years, using the *genepop* package (Rousset, 2008). All statistical analyses were conducted in R 4.1.9 using the *base* and *stats* packages (R Core Team, 2021).

RESULTS

Fluid Group Membership

Over the course of spring 2021, we marked a total of 75 unique female bees at 25 focal nests. We estimate that there were approximately 147 female bees residing in the log at this time based on the estimated ratio of marked to unmarked bees. Further, we estimate that there were approximately 40 active nests over the spring observation period. Of the 75 marked female bees, we observed 47 bees on more than one occasion, with an average of 2.57 ± 0.22 (range: 1–12) observations per individual. The bees that were only observed once may have dispersed to nests other than our 25 focal nests (including nests in other, distant aggregations) or may not have left the nest during our chosen sampling times. Of the 47 bees observed more than once, 16 (34.04%) were observed only at a single nest. The remaining 31 bees (66.96%) were observed at multiple nests: 19 bees (40.43%) were observed at 2 different nests, 7 bees (14.89%) were observed at 3 different nests, 3 were bees (6.38%) observed at 4 different nests, and 2 bees (4.26%) were observed at 5 different nests (**Figure 1**). No bee was observed re-visiting a nest she had previously occupied.

Importantly, the number of nests visited by each bee is likely to be greater than what we were able to observe during this limited observation period. Many of the 28 bees we marked but did not recapture may have relocated to non-focal nests. For all bees observed more than once, we observed a significant correlation between number of observations of each bee and the observed number of nests visited (r = 0.49; df = 44, P < 0.001). This correlation suggests that more intensive sampling would reveal even lower nest fidelity.

Relatedness Within and Between Groups

We sampled genetic material from 68 adult females in spring 2019 and 2020. In 2019 we sampled 29 females from 12 nests and in 2020 we sampled 39 females from 18 nests. We sampled between 1 and 7 females per nest (mean = 2.27, S.E. = 0.28). Population genetic analyses showed little genetic differentiation between years (across loci F_{ST} = 0.0488; **Supplementary Table 2**). Hardy-Weinberg equilibrium was verified for all but two loci (XS7 and XS30; **Supplementary Table 2**). In XS7 F_{IS} analysis showed an extreme overabundance of heterozygotes (F_{IS} = −0.97). We calculated the relative relatedness of all pairwise comparisons of individuals sampled (Queller and Goodnight, 1989). In this metric of relatedness, a value of 0 refers to the average relatedness of all individuals sampled. Positive values refer to above-average relatedness and negative values refer to below-average relatedness. A relative relatedness value of 1 indicates that the two individuals share alleles at all six loci tested. In 2019, the estimated relatedness (r) of nestmates (mean = −0.09, SE = 0.15, median = 0.10) was indistinguishable from the relatedness of non-nestmates (mean = 0.10, S.E. = 0.03,

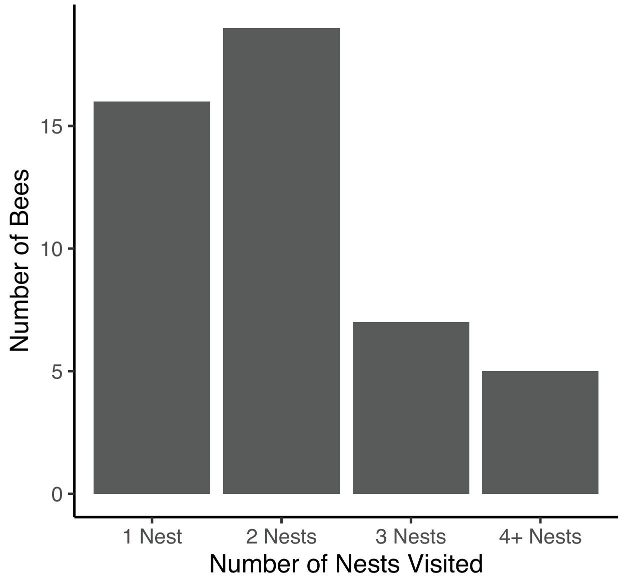

FIGURE 1 | Counts of uniquely identified bees observed at 1, 2, 3, or ≥ 4 nests over the course of the 2021 reproductive season.

median = −0.023; Wilcoxon test: P = 0.500; **Figure 2**). In 2020, the relatedness of nestmates (mean = 0.35, S.E. = 0.07, median = 0.336) was significantly higher than the relatedness of non-nestmates (mean = −0.05, S.E. = 0.02, median = −0.01; Wilcoxon test: $P < 0.001$; **Figure 2**).

DISCUSSION

Choosing whom to live with is one of the most consequential social decisions animals make. Kinship can factor strongly into this decision when individuals receive indirect fitness benefits from helping relatives reproduce. When helping behavior is limited, as for many carpenter bees (Gerling et al., 1989; Richards, 2011; Prager, 2014), incentives for nesting with kin may be likewise minimal. We explored nesting decisions in the valley carpenter bee (*X. sonorina*), which face severe intraspecific competition over nesting opportunities (Gerling, 1982; Ostwald et al., 2021). We demonstrated that nest joining is common throughout the reproductive season in this species. Joining behavior may prompt associations between non-relatives. Indeed, we present genetic evidence suggesting a mix of kin and non-kin in nesting groups, with many close relatives nesting apart, and many unrelated individuals nesting together. These highly dynamic social groups raise important questions about the costs and benefits of group living in different social contexts.

Inter-nest migration creates opportunities for individuals to associate with non-relatives. Often, these movements reflect adaptive strategies to access limited reproductive opportunities through resource sharing or cooperation, as in many communal birds and insects (Abrams and Eickwort, 1981; Wcislo, 1993; Vehrencamp, 2000; Wcislo and Tierney, 2009; Riehl, 2011). In other cases, relocation may reflect usurpation (Klahn, 1988; Hogendoorn and Leys, 1993). In our study, a majority of bees (67%) relocated from the nests at which they were originally captured (similar to rates observed in other Hymenopteran species; *Megachile rotundata*, Goerzen et al., 1995; *X. virginica*, Peso and Richards, 2011; *Polistes canadensis*, Sumner et al., 2007), and 27% relocated more than once. These moves may be temporary or permanent, reflecting both relocation and perhaps inspection of possible nesting sites. Notably, however, we never observed a bee relocate and then return to her previous nest, suggesting that relocations are often long-term.

The high rates of nest relocation in our study likely represent attempts to seek out reproductive opportunities within saturated nesting space. Bees that relocate may be attempting to usurp dominant reproductives in nearby nests (Hogendoorn and Leys, 1993; Hogendoorn, 1996; Richards, 2011). Alternatively, they may join existing groups as subordinates, but perhaps with a greater chance of nest inheritance than they had in their natal nests (Richards and Course, 2015). Notably, nest relocation was common despite the fact that intruders are usually treated aggressively by resident bees (Velthuis and Gerling, 1983; Hogendoorn and Velthuis, 1995), suggesting that the potential benefits of relocation can outweigh the costs of physical conflict. The fitness outcomes of the relocation strategy compared with remaining in the natal nest remain to be investigated.

Often termed "drifting," nest relocation behavior should not be conflated with navigational errors. For example, navigational errors are a well-documented apicultural phenomenon in which honey bees enter unfamiliar hives in crowded apiaries (Free, 1958; Pfeiffer and Crailsheim, 1998; Oliveira et al., 2021). Studies of inter-nest movements in the paper wasp *Polistes canadensis* and the eastern carpenter bee *X. virginica* found evidence that nest relocation in these species was not merely the result of navigational errors (Sumner et al., 2007; Peso and Richards, 2011). In a study of the navigational abilities of *X. sonorina*, females made very few navigational errors, even in treatments designed to disrupt homing cues (Ostwald et al., 2019). These observations, coupled with the high incidence (67%) of nest relocation in our study, suggest that nest relocation here represents an active strategy rather than simply a consequence of navigational errors.

Our behavioral data support the results of our genetic analysis, which suggests that nestmates are not always close relatives. In 2019, we found that nestmates were no more related to one another than they were to non-nestmates. Frequent inter-nest migration, as observed in our mark-recapture data, was likely to be the mechanism diluting relatedness in these nestmate groups. In 2020, however, we found nestmates to be significantly more related than non-nestmates, despite sampling at the same time of year across sampling years. This suggests that levels of relatedness vary, across years and likely seasonally. If females overwinter with siblings, then we would expect relatedness to progressively decrease over the reproductive season, as bees disperse and are driven from the nest by dominant bees (Velthuis, 1987; Richards and Course, 2015; Vickruck and Richards, 2021). Also, annual variation in the timing of environmental cues regulating carpenter bee social phenology (Minckley, 1987;

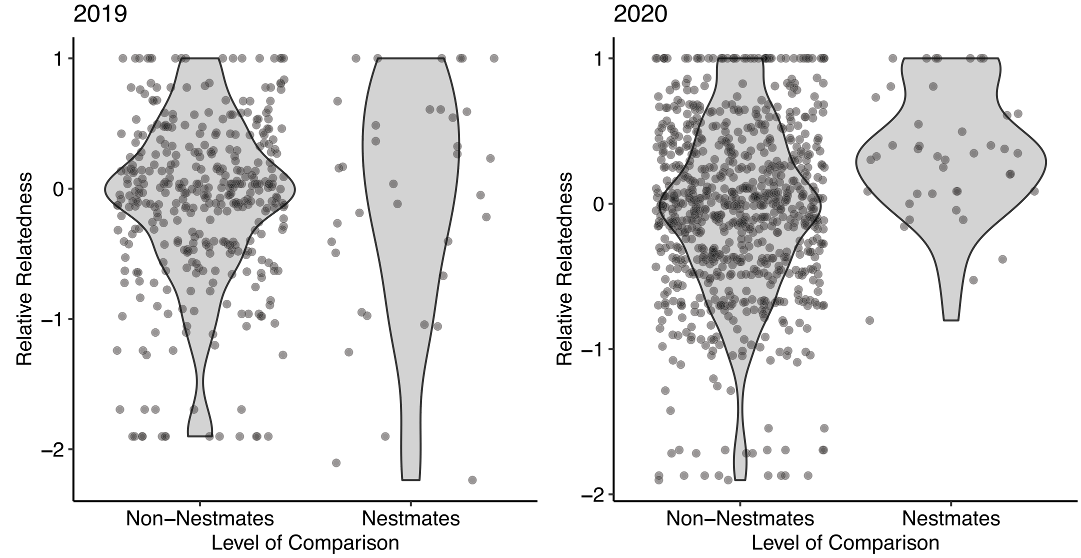

FIGURE 2 | Estimated relative relatedness (*r*) of nestmate vs. non-nestmate females in a single nesting aggregation. Each point represents a single pairwise comparison between two unique individuals in the sample. In 2019 (left), nestmates were no more related than non-nestmates (Wilcoxon test: $P = 0.500$). In 2020, nestmates were significantly more related than non-nestmates (Wilcoxon test: $P < 0.001$). Shaded areas indicate probability density.

Ostwald et al., 2020) could account for observed differences across years if, for example, bees began foraging and dispersing later in 2020 than in 2019.

Alternatively, the extent of dispersal and nest relocation across years may depend on factors such as population density and the degree of intraspecific competition. Further sampling throughout the year and across years would usefully clarify the extent to which relatedness changes over time and how these patterns are shaped by environmental factors. Our observed differences in relatedness may be, in part, an issue of limitations on genetic markers. We examined genetic loci characterized for another species, *X. virginica* (Vickruck, 2015), which we demonstrated to be present and variable in *X. sonorina*. However, developing species-specific genetic markers will enable greater resolution of relatedness estimates in future studies.

Why might bees leave their natal nests to join individuals to which they are not closely related? Many non-kin groups benefit from task sharing that improves survival or fitness by reducing the individual labor burden (Bernasconi and Strassmann, 1999; Tibbetts and Reeve, 2003; Cahan and Fewell, 2004; Wilkinson et al., 2016). Carpenter bees, however, do not share the labor costs of reproduction, with only the reproductively active female contributing meaningfully to foraging and nest construction (Richards, 2011; Richards and Course, 2015). As such, additional group members may not improve the productivity of the nest (Prager, 2014; Buchmann and Minckley, 2019). In the absence of helping behavior, the indirect fitness benefits of remaining in the natal nest with relatives are likely to be low or absent. Instead, females may prioritize seeking direct fitness opportunities wherever they may be available, with kin or non-kin.

At the same time, social decisions may not necessarily be made irrespective of kinship. Temporary matrifilial societies may arise from generation overlap between mothers and recently emerged offspring, in which offspring may guard the nest and receive food from their mother (Gerling, 1982; Gerling et al., 1983; Velthuis and Gerling, 1983). Conversely, non-kin nesting may actually represent a strategy to maximize reproductive opportunities among kin. Data from *X. virginica* even suggest that females may actively avoid nesting with relatives during the reproductive season to reduce kin competition (Vickruck and Richards, 2021). Indeed, our data show many instances of closely related non-nestmates. Further study is needed to determine whether females can benefit from associating with relatives, and if so, what conditions and life history stages favor these associations.

In conclusion, we found evidence for variable relatedness within carpenter bee nesting groups, suggesting that groups can consist of a dynamic mix of kin and non-kin nestmates. This study represents one of only two to quantify genetic relatedness in *Xylocopa* groups (Vickruck and Richards, 2021). Observations of nest relocation in an additional two *Xylocopa* species suggests that low relatedness may be common among the social species in the genus *(X. pubescens*, Gerling et al., 1983; *X. sulcatipes,* Velthuis, 1987). Nest membership in our study was highly fluid, with most females spending time in multiple nests over the course of the reproductive season. This nest relocation strategy likely reflects attempts to secure reproductive opportunities among strongly limited nest sites. Changes in nest membership demonstrate that relatedness is not a fixed condition, but rather may shift with seasonal and social variables. Instances of low relatedness among our sampled bees suggest limited indirect fitness benefits for nestmates, and instead emphasize the importance of ecological factors, especially nesting constraints, in facilitating sociality in *X. sonorina*

(Ostwald et al., 2020, 2021). Though sociality is often interpreted through the lens of kin selection, systems such as these with low and dynamic relatedness highlight the complexity of social decisions beyond the role of kinship.

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

MO collected the data and wrote the manuscript. MO and RD analyzed the data. All authors contributed to study design and manuscript editing.

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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.2021.767380/full#supplementary-material

Supplementary Table 1 | Amplified microsatellite loci with sequence, dye, and multiplex information. Loci were characterized for *X. virginica* in Vickruck, 2015.

Supplementary Table 2 | Summarized population genetic information across microsatellite loci, including F_{ST}, F_{IS}, X^2 test for Hardy-Weinberg Equilibrium with associated DF and P-values.

Supplementary Data Sheet 1 | Queller-Goodnight estimated relatedness values for all comparisons within and between colonies of the focal population presented in the paper, as well as for non-focal populations ("BetweenPop" comparisons).

Supplementary Data Sheet 2 | Mark recapture raw data including date of capture and nest identity.

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Selection Forces Driving Herding of Herbivorous Insect Larvae

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Herding behavior is widespread among herbivorous insect larvae across several orders. These larval societies represent one of several different forms of insect sociality that have historically received less attention than the well-known eusocial model but are showing us that social diversity in insects is broader than originally imagined. These alternative forms of sociality often focus attention on the ecology, rather than the genetics, of sociality. Indeed, mutually beneficial cooperation among individuals is increasingly recognized as important relative to relatedness in the evolution of sociality, and I will explore its role in larval insect herds. Larval herds vary in in the complexity of their social behavior but what they have in common includes exhibiting specialized social behaviors that are ineffective in isolated individuals but mutually beneficial in groups. They hence constitute cooperation with direct advantages that doesn't require kinship between cooperators to be adaptive. Examples include: trail following, head-to-tail processions and other behaviors that keep groups together, huddling tightly to bask, synchronized biting and edge-feeding to overwhelm plant defenses, silk production for shelter building or covering plant trichomes and collective defensive behaviors like head-swaying. Various selective advantages to group living have been suggested and I propose that different benefits are at play in different taxa where herding has evolved independently. Proposed benefits include those relative to selection pressure from abiotic factors (e.g., thermoregulation), to bottom-up pressures from plants or to top-down pressures from natural enemies. The adaptive value of herding cooperation must be understood in the context of the organism's niche and suite of traits. I propose several such suites in herbivorous larvae that occupy different niches. First, some herds aggregate to thermoregulate collectively, particularly in early spring feeders of the temperate zone. Second, other species aggregate to overwhelm host plant defenses, frequently observed in tropical species. Third, species that feed on toxic plants can aggregate to enhance the warning signal produced by aposematic coloration or stereotyped defensive behaviors. Finally, the combination of traits including gregariousness, conspicuous behavior and warning signals can be favored by a synergy between bottom-up and top-down selective forces. When larvae on toxic plants aggregate to overcome plant defenses, this grouping makes them conspicuous to predators and favors warning signals. I thus conclude that a single explanation is not sufficient for the broad range of herding behaviors that occurs in phylogenetically diverse insect larvae in different environments.

Keywords: caterpillars, cooperation, aggregation, group-living, gregarious, thermoregulation, social facilitation of feeding, aposematism

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COOPERATION IN LARVAL HERDS

Cooperation is said to occur when the behavior of one individual benefits others (West et al., 2021). This tends to lead to grouping as individuals stick together in order to accrue benefits from the actions of their neighbors. The essence of sociality has been described as "reciprocal communication of a cooperative nature" (Wilson, 1971), since, by definition, an individual can only incur benefits from remaining in a group if others remain there as well.

Kin selection has historically been considered the driving force in insect sociality, whereby individuals reap indirect fitness benefits by helping closely related kin. However, recent work has challenged this paradigm (Nowak et al., 2010), suggesting that direct fitness benefits can drive cooperation in insect groups, even among classically eusocial Hymenoptera (Hölldobler and Wilson, 2008; Leadbeater et al., 2011; Brahma et al., 2019). Direct benefits from natural selection that don't require kinship between cooperators are thus receiving increasing attention as an alternative framework for understanding insect sociality (Leimar and Hammerstein, 2010; West et al., 2021).

The broad diversity of social forms across multiple insect species provides valuable insights into the evolution and ecology of sociality beyond the hymenopteran eusocial continuum (Choe and Crespi, 1997; Costa, 2006; Rubenstein and Abbot, 2017); these have evolved independently and are likely driven by different selection pressures. This paper examines one phylogenetically widespread "alternative" form of insect sociality, herding of herbivorous insect larvae. This describes larval insects that aggregate and use a variety of mechanisms to remain together on their host plant, often gradually dispersing as they grow larger and becoming solitary as adults. Larval herds lack parent-offspring interaction and often include unrelated individuals (Costa, 2018) and thus challenge us to think beyond the eusocial model when considering insect sociality. They can provide unique insights into ecological drivers of sociality.

Herds often arise from the same egg mass and thus can be made up of siblings, suggesting the possibility for indirect benefits *via* kin selection. Indeed, cooperation is considered more likely to occur when population structure favors grouping of related individuals, due to indirect benefits that accrue from cooperating with relatives (Wilson, 1971). However, cooperation can be favored by natural selection if it is mutually beneficial and directly benefits the actor as well as recipients, whether they are related or not. Cooperation only appears paradoxical if it incurs a cost to the cooperator, which is not always the case (Leadbeater et al., 2011; Brahma et al., 2019). Indeed, recent work shows strong evidence for substantial direct benefits of cooperation in multiple animal taxa (West et al., 2021). Thus, although kin selection could play a role in favoring cooperation in larval herds, it is not necessarily required and direct benefits could provide more parsimonious explanations (Nowak et al., 2010). Existing evidence suggests that kin selection is not essential to promote herding: group mixing occurs in the few species in which the genetic structure of herds has been investigated, and no species studied to-date show any evidence of kin recognition (Costa, 2018).

This paper examines benefits of grouping in larval herds taking a direct fitness perspective, i.e., examining advantages to the individual of staying in the group vs. leaving. This approach remains neutral as to whether kin selection is involved, and focuses instead on the natural selection drivers of social behaviors. These occur independently of any putative indirect benefits. Evidence suggests that individual larvae weigh costs and benefits of remaining with the group and do leave herds when remaining becomes costly (Plenzich and Despland, 2018). Larval herding thus appears to be an instance where cooperation is mutually beneficial and hence where direct fitness benefits play an important role.

NATURAL HISTORY OF LARVAL HERDS

Larval gregariousness is observed in many insect species across several orders (Costa, 2006). The best studied species are Lepidopteran caterpillars, but examples are also known among sawfly (Hymenoptera) and beetle (Coleoptera) larvae and grasshopper (Orthoptera) nymphs. Many of these species clearly actively aggregate, rather than merely staying together following hatching on a high quality food source. There is no evidence for kin recognition in those species where it has been studied [caterpillars (Costa and Louque, 2001; Costa and Ross, 2003; Sun and Underwood, 2011) and sawflies (Terbot et al., 2017)]. Division of labor has been suggested in some species (Ghent, 1960; Underwood and Shapiro, 1999), but was not detected in others (Costa and Ross, 2003; McClure et al., 2011b) and does not seem to play a major role.

The mechanisms used by individuals to remain with the group have been studied in detail in several species, showing a range of sophisticated forms of communication whose main purpose appears to be keeping the group together (Despland, 2013). The best-known is pheromone trail following (see https://alisonloader.com/mass-transit/ for an artist's manipulation of trail-following caterpillars by drawing artificial pheromone trails). This mechanism has been documented in caterpillars (Peterson, 1988; Roessingh, 1989; Fitzgerald, 1993b,a; Fitzgerald and Underwood, 1998; Ruf et al., 2001; Costa and Gotzek, 2003; Fitzgerald and Pescador-Rubio, 2011; Pescador-Rubio et al., 2011), beetle (Fitzgerald et al., 2004) and sawfly larvae (Costa and Louque, 2001). Other mechanism to maintain group cohesion include allomimesis (Despland et al., 2017) and processions (Fitzgerald, 2003) in caterpillars, and synchronization of movement (Despland and Simpson, 2006; Despland, 2020) in grasshopper nymphs. The existence of these behaviors underscores the benefits of cooperation, since they have clearly been shaped by natural selection to ensure that individuals do not get separated from the group (Hofmann et al., 2014).

Herding larvae exhibit various forms of social organization, generally categorized by different modes of foraging. Some larvae exhibit patch restricted foraging whereby the herd forms a shelter, often by spinning silk, and feed on the foliage enclosed within the shelter. Others are nomadic, using pheromone trails or other cues to move together between feeding sites. Some of the best studied species are central place foragers that build a

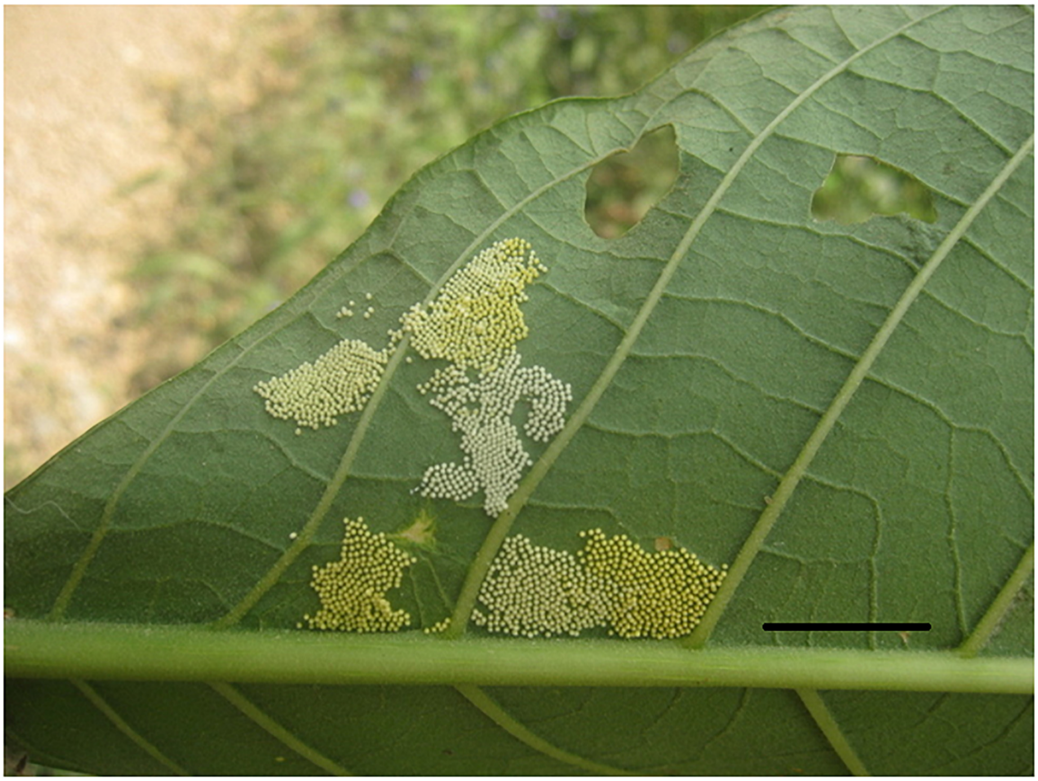

FIGURE 1 | Multiple clusters of *Ithomia* spp., eggs on a single leaf. Black bar indicates 1 cm.

shelter (again often using silk) then move out of the shelter to find food sources (Costa and Pierce, 1997; Fitzgerald and Costa, 1999). Broadening phylogenetic and geographic scope has shown myriad variations on these themes, including species that change between different organizational structures during larval development (Costa, 2006).

Most larval herds begin as sib-groups that emerge from the same egg mass, but fail to disperse. There are many reasons why herbivorous female insects might lay eggs in clusters on host plants, including constraints on the adult female (e.g., difficulty in host finding, short longevity), benefits to the eggs (e.g., protection from freezing or desiccation) and grouping of larval progeny once they emerge from the egg (Stamp, 1980). However, in many species that lay eggs in clusters, the larvae disperse upon hatching, suggesting that larval gregariousness can be selected for separately from egg-clustering.

At high population densities, larvae from different egg masses can fuse into large herds of multiple sib-groups (Costa and Ross, 2003; Fletcher, 2009). Indeed, some species preferentially lay eggs close to conspecific egg-masses (see **Figure 1**), presumably in order to increase group size (Stamp, 1980; Codella and Raffa, 1993). In the single species studied, this was shown to be adaptive due to the increase in group size despite the dilution of relatedness (Costa and Ross, 2003). Groups are often most cohesive early in caterpillar ontogeny, and caterpillars often disperse in the later instars, suggesting that benefits of grouping decrease as caterpillars grow larger (Despland and Hamzeh, 2004; Colasurdo and Despland, 2005; Despland and Huu, 2007). Herds can also dissolve under poor food conditions, as larvae move away to forage individually (Plenzich and Despland, 2018). The cost-benefit ratio of cooperation thus varies over larval ontogeny and in different environments (Guindre-Parker and Rubenstein, 2020).

Several different advantages to larval herding have been proposed in various insect species, and these can be grouped into broad categories based on the driving selection pressure: environmental pressures, bottom up forces from host plants, and top-down forces from natural enemies. I review these in the following sections and discuss contexts in which they might apply.

ENVIRONMENTAL DRIVERS: THERMOREGULATION

Insect larvae are poikilothermic and therefore suffer slower metabolism, growth and development at lower temperature. Several caterpillar species have been shown to reap thermal gains from collective basking (**Table 1** and **Figure 2**) under conditions of relatively low temperature but high solar radiation. This cooperative thermoregulation sometimes includes the construction of a shelter or tent that can be used to further increase caterpillar body temperature (**Table 1**).

TABLE 1 | List of insect species in which collective larval thermoregulation has been recorded, including the location of the study, the insect species involved and the family to which it belongs (all in the order Lepidoptera unless stated otherwise), larval coloration, presence of setae and of tent building behavior.

Study	Location	Species	Family	Color	Setae?	Tent?	Phenology	Gain (°C)	Ambient temperature
McClure et al., 2011	Quebec, Canada	*Malacosoma disstria*	Lasiocampidae	Black	Yes	No	Early spring	4	18
Knapp and Casey, 1986	New Jersey, United States	*Malacosoma americanum*	Lasiocampidae	Black	Yes	Yes	Early spring	18	10
Ruf and Fiedler, 2002	Bavaria, Germany	*Eriogaster lanestris*	Lasiocampidae	Black	Yes	Yes	Spring	18	16
Ruf et al., 2003	Bavaria, Germany	*Eriogaster catax*	Lasiocampidae	Black	Yes	No	Spring	N/A	N/A
Ruf et al., 2003	Bavaria, Germany	*Malacosoma neustria*	Lasiocampidae	Black	Yes	Yes	Spring	N/A	N/A
Battisti et al., 2013	Gotland, Sweden	*Thaumetopoea pinivora*	Notodontidae	Yellow	Yes	No	Spring	7	9
Ronnas et al., 2010	Gotland, Sweden	*Thaumetopoea pinivora*	Notodontidae	Yellow	Yes	No	Spring	6	10
Frid and Myers, 2002	Vancouver, Canada	*Malacosoma pluviale*	Lasiocampidae	Black	Yes	Yes	Spring	21	16
Stamp and Bowers, 1990	Massachusetts, United States	*Hemileuca lucina*	Saturniidae	Black	Yes	No	Spring	5	20
Seymour, 1974	Victoria, Australia	*Perga dorsalis*	Pergidae (Hymenoptera)	Blue-black	No	No	Austral spring	N/A	N/A
Fletcher, 2009	Canberra, Australia	*Perga affinis*	Pergidae (Hymenoptera)	Black	No	No	Austral winter	13	N/A
Porter, 1982	Oxford, United Kingdom	*Euphydryas aurinia*	Nymphalidae	Black	Yes	No	Spring	20	15

The table also indicates the season in which the thermoregulatory behavior is observed, the maximum thermal gains obtained (difference °C between larvae and ambient) and the temperature (°C) at which measurements are made.Many other studies suggest thermoregulatory benefits to grouping but only those in which thermal gains are explicitly recorded are included here.

Larval grouping has also been suggested to facilitate physiological regulation by preventing water loss. Improved water balance has been shown in aggregations of *Imbrasia belina* (Westwood) (Saturniidae) caterpillars in South Africa (Klok and Chown, 1999) and *Chlosyne lacinia* (Geyer) (Nymphalidae) in Arizona (Clark and Faeth, 1997), as well as within the tents of *Inachis io* (Linnaeus, 1758) (Nymphalidae) in the United Kingdom (Willmer, 1980). However, no discernable effect of aggregation was shown on water loss in *Eutrichia capensis* (Lasiocampidae) in South Africa (Schoombie et al., 2013). It has been noted that insects feeding on foliage (which always has a high water content) are not likely to face great risk of desiccation except during periods of food deprivation (Klok and Chown, 1999).

Further investigation of species that show thermal gains in aggregations have shown that caterpillars can modulate their grouping behavior depending on ambient conditions. Caterpillars move to a basking spot under a heat lamp at low temperatures but not at high ones, and aggregation is tightest under conditions where it is most beneficial (low temperature and high solar radiation) (McClure et al., 2011a). Tent-builders move around inside the tent during the day to optimize temperature (Joos et al., 1988; Ruf and Fiedler, 2002; Ruf et al., 2003).

Many of the species that bask collectively to elevate body temperature are early spring feeders of the temperate zone (**Table 1**). These caterpillars hatch in early spring to feed on expanding foliage, which is generally softer and more nutritious than mature foliage (Despland, 2018) and to use the enemy-free space before many predators become active (Parry et al., 1998). However, these caterpillars emerge when temperatures are below optimal for growth and development, even below freezing (Despland, 2021), and many show adaptations that increase thermal gains from radiant solar energy: dark color, dense setae (Casey and Hegel, 1981), and collective basking.

A few notable well-documented biogeographical outliers include species that are active during winter in cool regions (*Thaumatopoea pityocampa* (Notodontidae) in the Mediterranean and *Eucheira socialis* (Pieridae) in Mexico) and cooperate to build tents to maximize solar radiation (Fitzgerald and Underwood, 2000; Uemura et al., 2020). As in the early-spring feeders above, these caterpillars are active at low, even below-freezing, temperatures, when cooperative thermoregulation is most advantageous.

Thermoregulation thus seems to have played an important role in shaping the biology of cold-weather active caterpillars, including their cooperative basking and shelter-building behavior (Joos et al., 1988; Joos, 1992; Despland, 2013). The best-studied among these are early-spring feeders in temperate-zone-inhabiting members of the Lasiocampidae. Collective thermoregulatory behaviors are often associated with other traits like dark pigmentation and dense setae that also improve heat capture; cooperative thermoregulation is thus part of an adaptive suite of traits associated with the niche of early spring feeding that is particularly common among the Lasiocampidae. There are close to 2,000 species in the family; it is not known how many of these have gregarious larvae, nor are the phylogenetic

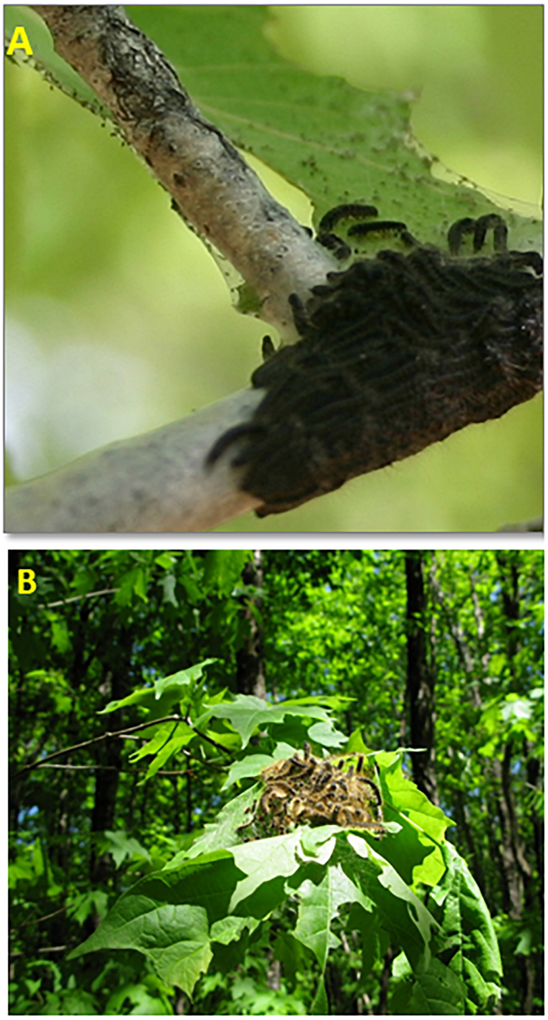

FIGURE 2 | Thermoregulatory cooperation: *Malacosoma disstria* **(A)** second instar (body length 1–1.5 cm) and **(B)** fifth instar (body length > 5 cm) caterpillars basking collectively to increase body temperature in the boreal forest of Québec, Canada.

relationships between them clear (Regier et al., 2000; Zolotuhin et al., 2012). It has been suggested that gregarious larvae have evolved three separate times within the Lasiocampidae (Regier et al., 2000), but clearly much remains to be understood about the evolution of larval cooperation in this family and the role played by cooperative thermoregulation.

BOTTOM-UP FORCES

Efficiency of Foraging

Information sharing to optimize nutritional intake is thought to be a major driver of the evolution of sociality (Giraldeau and Caraco, 2000; Rubenstein and Abbot, 2017; Lihoreau et al., 2018). Collective foraging based on recruitment to pheromone trails is well-known to improve efficiency of food finding and exploitation by ants (Wilson, 1971; Hölldobler and Wilson, 2008). It therefore tends to become the default expectation for gregarious insects, especially those that use pheromone trails. Efficient collective foraging implies that individuals who find food recruit their colony-mates to the food source, and that strength of recruitment is modulated by food quality such that individuals are preferentially recruited to better quality sources (Dussutour et al., 2007; Lihoreau et al., 2018). Gregarious caterpillars, weevil and sawfly larvae use pheromone-marked silk trails to direct locomotion, but it is by no means evident that these trails improve the efficiency of foraging. Consistent choice of the better quality food source has only been demonstrated in the central-place foraging Lasiocampids *Malacosoma americanum* (Fitzgerald and Edgerly, 1979; Fitzgerald and Peterson, 1983; Fitzgerald, 1995) and *Eriogaster lanestris* (Ruf et al., 2001). By contrast, when a herd of the nomadic *Malacosoma disstria* are presented with a choice between two food sources, the entire group generally remains cohesive and moves together to one of the sources (Dussutour et al., 2008). The entire herd exploits whichever food source was discovered first (Dussutour et al., 2007), and often the second source isn't even sampled.

Indeed, *M. disstria* have been shown to trade-off selectivity in foraging for the advantages of staying together (Santana et al., 2015). Similarly, gregarious grasshopper nymphs [*Chromacris psittacus* (Romaleidae)] have been shown to remain feeding on the same leaf rather than sampling multiple leaves and exhibiting choice like the solitary adults of their species (Despland, 2020). Mathematical models suggest that cooperation *via* information sharing can improve individual foraging success when food is scarce and scattered (e.g., eusocial hymenopterans, seabirds), but that social interactions do not improve individual foraging when it is abundant and scattered, as is generally the case for herbivores (Giraldeau and Caraco, 2000; Rubenstein and Abbot, 2017). It seems that instead grouping imposes constraints on foraging, as it requires individuals to maintain contact and exchange information in order not to become separated from each other (Santana et al., 2015). These constraints can be minimized in central-place foragers by selective recruitment based on food quality, as occurs in *M. americanum* and *E. lanestris*. However, although selective recruitment has only been investigated in a few species, it does not appear to be widespread. Instead, increased costs associated with collective foraging constraints occurring under food limitation can lead to individuals ceasing to cooperate and to the break-up of groups (Plenzich and Despland, 2018).

Overcoming Plant Defenses

Another way in which gregarious insect larvae can cooperate is in overcoming plant defenses, either physical or chemical (see **Figure 3**). Indeed, herbivorous insects and plants engage in an evolutionary arms race, in which plants mount an array of defenses, including constitutive and inducible production of toxic, distasteful and/or glue-like compounds, toughness of foliage, trichomes on leaves to act as a mechanical barrier to small insects and trichomes containing toxic compounds to poison insects before they take their first bite. Herbivorous insects exhibit countermeasures, including various detoxification enzymes, sequestration of plant compounds, strong mandibles,

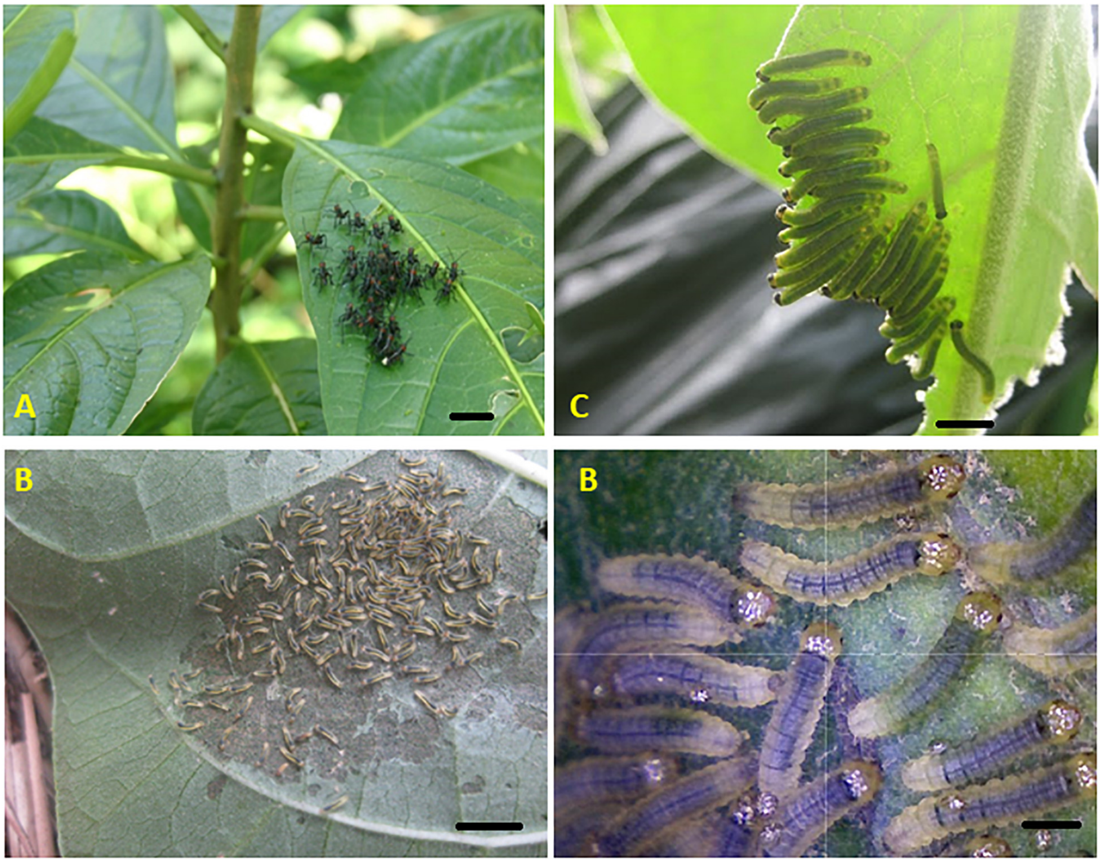

FIGURE 3 | Cooperation in overcoming plant defenses (bottom-up pressures): **(A)** synchronized feeding by *Chromacris psittacus* nymphs (Romaleidae), **(B)** collective leaf windowing by *Pagyris cymothoe* larvae, and **(C)** edge feeding by *Ithomia* larvae (both Ithomiini). All three species observed in secondary vegetation in cloud forest, Ecuador. Black bar in each panel indicates 1 cm.

and even tarsal claws for climbing over trichomes (Despres et al., 2007). Gregarious larvae also use collaborative behaviors in response to plant defenses.

Multiple studies have shown that herbivorous insects reared at optimal temperatures in the absence of natural enemies grow faster and survive better in groups than alone (see **Table 2**), and have suggested various possible mechanisms for density-dependent manipulation of host quality by which grouping facilitates feeding on defended host plants. One possible mechanism is synchronous feeding to outpace and overwhelm production of induced chemical defenses (Denno and Benrey, 1997). Indeed, induced defenses are activated in plant tissues in response to herbivore biting, but this process takes time; hence herbivores can avoid these toxic compounds by feeding together on one leaf until induced defenses appear, then moving away on to an undamaged leaf (de Bobadilla et al., 2021). Mathematical modeling shows that this time-lag in induced defenses can lead mobile herbivores to aggregate, feed synchronously and move from induced to undamaged plant parts (Anderson et al., 2015). Other mechanisms by which insect larvae can collectively feed on plants inaccessible to isolated individuals include working together to initiate a feeding edge on tough foliage (Ghent, 1960; Nahrung et al., 2001), and collectively laying down silk to move over glandular trichomes without contacting the heads that contain toxins (Young and Moffett, 1979; Despland and Santacruz, 2020).

Social facilitation of feeding has been less well studied in gregarious larvae than has thermoregulation, but it also seems more prevalent in young larvae than in older larvae, presumably because larger individuals are better equipped to handle plant defenses. For instance, smaller larvae have smaller mandibles and are less able to chew tough leaves (Clissold, 2008; Nishida, 2010), are smaller relative to plant structures like trichomes (Despland and Santacruz, 2020), and have less well developed detoxification enzymes to handle plant defensive compounds (Despres et al., 2007).

Social facilitation of feeding on defended plants has also been observed more frequently in the tropics than has thermoregulation (**Table 2**), suggesting that benefits of cooperation in larval herds differ between environments. Indeed, the environmental factors that drive collective thermoregulation are often thought to be more limiting in high-latitude environments (Dobzhansky, 1950; Schemske, 2009). Conversely, trophic relationships and interspecific interactions

TABLE 2 | List of studies demonstrating social facilitation of feeding, indicating the location and biome in which the study was conducted, the species and family of insect involved, the family of the plant on which assays were conducted and the reported mechanism.

Study	Location	Biome	Species	Family	Host plant	Mechanism
Allen, 2010; Nishida, 2010	Costa Rica	Rainforest	*Euselasia chrysippe*	Riodinidae	Melastomataceae	Feeding facilitation
Chang and Morimoto, 1988	Japan	Temperate deciduous	*Gastrolina depressa*	Coleoptera Crysomelidae	Juglandaceae	Overcoming leaf toughness
Clark and Faeth, 1997	Arizona, United States	Desert	*Chlosyne lacinia*	Nymphalidae	Asteraceae	Overcoming toughness and trichomes
Denno and Benrey, 1997	Veracruz, Mexico	Rainforest	*Chlosyne janais*	Nymphalidae	Acanthaceae	Overwhelming induced chemical defenses
Despland, 2019	Mindo, Ecuador	Secondary growth, cloudforest	*Mechanitis menapis*	Nymphalidae, Ithomiini	Solanaceae	Silking trichomes
Despland, 2020	Mindo, Ecuador	Secondary growth, cloudforest	*Chromacris psittacus*	Orthoptera: Romaleidae	Solanaceae	Defensive chemistry
Fiorentino et al., 2014	Maryland, United States	Temperate deciduous forest	*Acharia stimulea*	Limacodidae	Fagaceae	Overcoming leaf toughness
Fordyce, 2003	California, United States	Chaparral	*Battus philenor*	Papilionidae	Aristolochiaceae	Overwhelming induced chemical defenses
Inouye and Johnson, 2005	Costa Rica	Secondary growth, tropical dry forest	*Chlosyne poecile*	Nymphalidae	Acanthaceae	Silk
Lawrence, 1990	Virginia, United States	Temperate deciduous forest	*Halisidota caryae*	Arctiidae	Fagaceae, Juglandaceae, Hamamelidaceae	Feeding facilitation
Mcmillin and Wagner, 1998	Arizona, United States	Subalpine forest	*Neodiprion autumnalis*	Hymenoptera: Diprionidae	Pinaceae	Feeding facilitation
Nahrung et al., 2001	Tasmania, Australia	Temperate moist forest	*Chrysophtharta agricola*	Coleoptera: Chrysomelidae	Myrtaceae	Initiating feeding on tough leaves
Pescador-Rubio, 2009	Jalisco, Mexico	Dry tropical forest	*Hylesia lineata*	Saturniidae	Erythroxylaceae, Sapindaceae, Salicaceae	Feeding facilitation
Rathcke and Poole, 1975	Maracay, Venezuela	Rainforest	*Mechanitis polymnia isthmia*	Nymphalidae, Ithomiini	Solanaceae	Silking trichomes
Reader and Hochuli, 2003	NSW, Australia	Dry sclerophyll forest	*Doratifera casta*	Limacodidae	Myrtaceae	Feeding facilitation
Tsubaki and Shiotsu, 1982	Kyushu, Japan	Temperate rainforest	*Pryeria sinica*	Zygaenidae	Celastraceae	Overwhelming induced chemical defenses
Young and Moffett, 1979	Costa Rica	Secondary growth, rainforest	*Mechanitis polymnia isthmia*	Nymphalidae, Ithomiini	Solanaceae	Silking trichomes
Ghent, 1960	Ontario, Canada	Boreal forest	*Neodiprion pratti*	Hymenoptera: Diprionidae	Pinaceae	Initiating feeding on tough leaves
Chang and Morimoto, 1988	Nagano, Japan	Temperate deciduous forest	*Gastrolina depressa*	Coleoptera: Chrysomelidae	Juglandaceae	Initiating feeding on tough leaves

When no specific mechanism is described in the paper, this is left as "feeding facilitation." All species in the order Lepidoptera unless otherwise mentioned.

are considered more limiting in the biodiverse tropics, where bottom-up pressures from the plants at lower trophic levels appear important drivers of larval cooperation.

TOP-DOWN FORCES

Anti-Predator Dilution Effect

When prey animals aggregate, this satiates predators and dilutes individual predation risk (Codella and Raffa, 1993). This simple anti-predator defense was demonstrated in *Neodiprion sertifer* (Diprionidae) and *M. disstria,* in behavioral assays showing higher individual survival rate when groups rather than isolated individuals were exposed to a variety of predators, including ants, spiders, stinkbugs and parasitoid wasps (Codella and Raffa, 1993; McClure and Despland, 2011). However, this simple form of cooperation can be overridden by social predators that cooperate themselves, including paper wasps (McClure and Despland, 2010) and ants (Despland and Lessard, in press). Because workers forage for the entire colony and recruit nestmates to food finds, they do not satiate and can deplete entire caterpillar herds.

Collective Anti-Predator Defenses

Several species of herding larvae exhibit stereotyped collective behaviors in response to predator attacks that can be effective at repelling different enemies. Perhaps the best known example

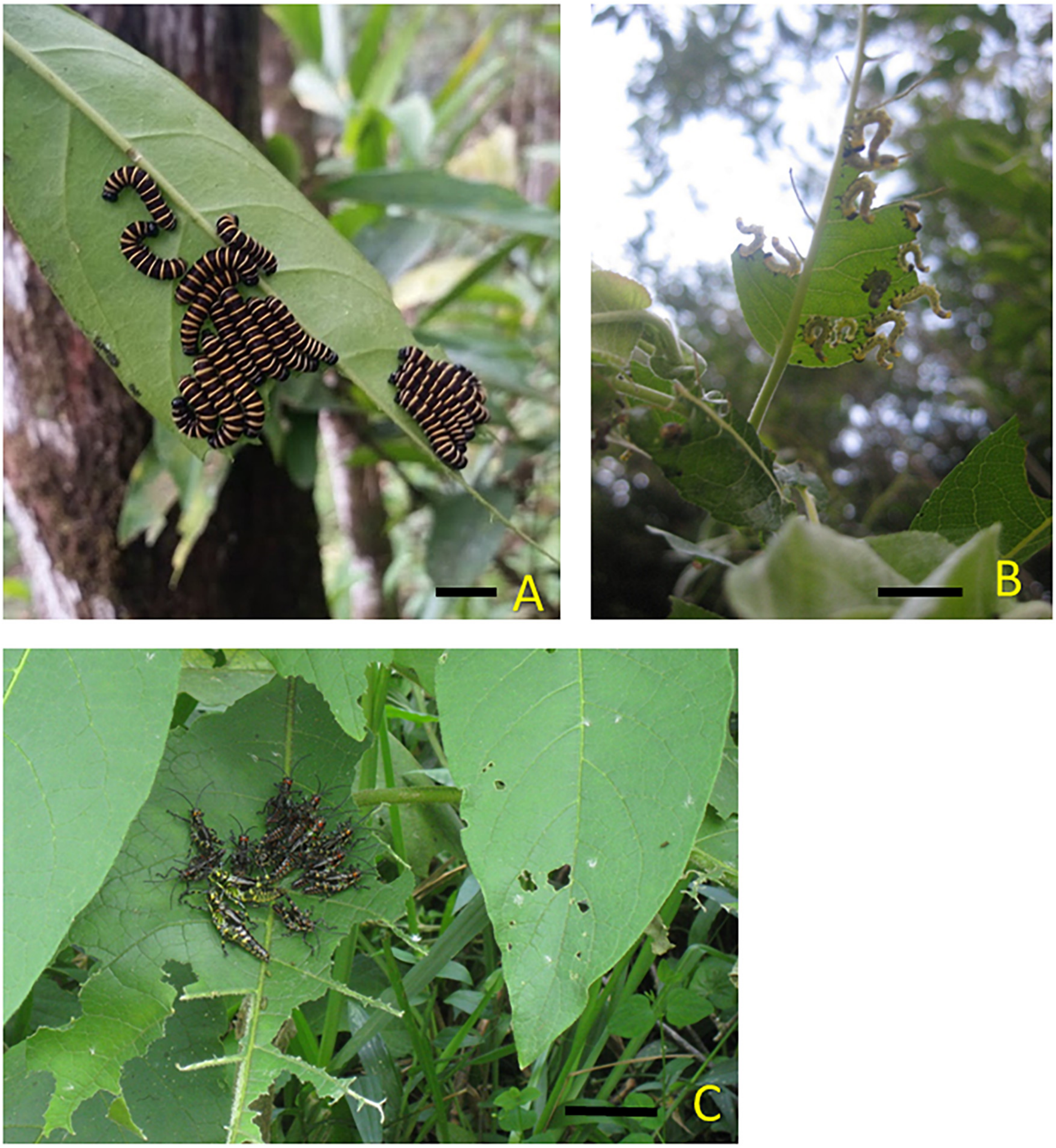

FIGURE 4 | Cooperation against predators (top-down pressures): **(A)** aposematic larvae of *Methona confusa* (Ithomiini) in secondary vegetation in cloud forest, Ecuador—photo credit Janeth Renteria, **(B)** collective defensive head-waving by *Nematus* spp., sawflies (Tenthredinidae) in boreal forest Québec, Canada, and **(C)** nymphs of *Chromacris psittacus* in secondary vegetation in cloud forest, Ecuador. Black bar in each panel indicates 1 cm.

is the collective display exhibited by gregarious sawflies (see **Figure 4**) in which individuals synchronously rear up, wave their heads and regurgitate on predators (Codella and Raffa, 1993; Fletcher, 2009). Another striking example is cycloalexy exhibited by sawfly, chrysomelid, weevil and fly larvae (possibly also caterpillars and thrips): individuals position themselves in a circle with defensive organs facing outward (Dury et al., 2014). These behaviors are paired with effective defenses, including regurgitation and/or toxin secretion, and can both directly repel predators and act as warning signals (Codella and Raffa, 1993).

Aposematism

Many gregarious larvae exhibit bright colors that can act as a warning signal to deter predators. Aggregation amplifies this warning signal and indeed many gregarious larvae have bright colors (see **Figure 4**). It has also been suggested that the stereotypical synchronized behavioral displays of sawflies further amplify the aposematic signal (Codella and Raffa, 1993). Larval coloration can thus provide some indication as to the form of cooperation underlying the evolution of gregarious behavior: larvae that aggregate to become more apparent to predators tend to be brightly colored, whereas larvae that aggregate to thermoregulate tend to be black.

Gregarious brightly-colored larvae have been documented in Lepidoptera, Symphyta (Hymenoptera), Orthoptera and Chrysomelidae (Coleoptera) (Costa, 2006). Coloration is generally interpreted as aposematic, although this can be difficult to test explicitly (Caro and Ruxton, 2019) since aposematism requires that the animal be toxic or somehow unprofitable to predators and that the color act as a signal to predators. Many of these insect larvae do feed on plants with powerful chemical defenses and some are known to sequester these plant compounds and to be toxic to predators. Gregarious aposematic species appear to be common in the tropics (Codella and Raffa, 1993; Costa, 2006), supporting the idea that interspecies interactions are important drivers of larval herd cooperation in tropical regions.

PATTERNS IN LARVAL COOPERATION

Phylogenetic and Biogeographical Patterns

Larval herds thus exhibit a range of cooperative behaviors in response to abiotic stresses as well as to both bottom-up and top-down biotic pressures. The best-studied cooperative behavior is cooperative thermoregulation, including tent building. This has been most frequently described in early spring feeders of the temperate zone. However, group living is also frequent in tropical insect larvae, and in those species that have been studied, the benefits of gregarious behavior seem mostly associated with overcoming plant defenses or protection from predators. Indeed, larval herding appears to have evolved more than once in over 300 insect families (Costa, 2006), and the underlying selection pressures likely differ between environments and life history strategies.

Considerable evidence exists documenting the costs and benefits of cooperation in individual species, but these can vary within species according to individual ontogeny or physiological state (Guindre-Parker and Rubenstein, 2020), between related species with different ecologies and life histories and across major biomes. Within individual herding insect species, cooperation often breaks down as larvae grow larger and benefits decrease but costs associated with competition and pathogen transmission increase (Despland, 2013), or under food limitation when individuals leave the group to forage independently (Plenzich and Despland, 2018). However, ecological determinants of the cost-benefit ratio of cooperation at the between-species level remain poorly understood (West et al., 2021). Thus, cost-benefit analyses could be applied across lineages like the Lasiocampidae, the Ithomiini (Nymphalidae), the Romaleidae and the Diprionidae that contain multiple species with gregarious larvae, exhibiting different group sizes, social organizations and individual color patterns. For instance, in the genus *Malacosoma* (Lasocampidae), some species are nomadic foragers (*M. disstria*) whereas others form tents (*M. americanum* and *M. californicum pluviale)*, despite the fact that thermoregulation appears to be the main selection pressure driving herding behavior in all these species (see **Table 1**). Similarly, within the Ithomiini, *Mechanitis menapis* and *Methona confusa* live in small groups of approximately 10 individuals whereas *Pagyris cymothoe* and *Ithomia* spp. form much larger aggregations (**Figures 1**, **3**, **4**), and some of these larvae show typical aposematic coloration while others appear more cryptic (**Figures 3**, **4**). Larval host plant specialization appears to have contributed to diversification in the Ithomiini (Willmott and Freitas, 2006), but the occurrence of larval herding across different species has not been documented or investigated in a phylogenetic context. One line of research to better understand the parallel evolution of larval cooperation would involve mapping patterns of social organization onto phylogenies of these taxa rich in gregarious larvae.

At the biogeographical level, broad patterns driving larval herding can be proposed: thermoregulation appears most important in the temperate zone, particularly in early spring- or even winter-feeders who face harsh abiotic conditions. By contrast, bottom-up and top-down biotic pressures more frequently drive larval cooperation in tropical species, in line with the long-standing theory that biotic interactions play the main role in driving evolutionary processes in the tropics (Dobzhansky, 1950; Schemske, 2009).

Cooperation in Integrated Suites of Traits

At the level of life history strategies, the best documented examples suggest that herding behavior is integrated within a suite of traits that together form a phenotype shaped by multiple selection pressures. One such adaptive suite of traits is seen in gregarious temperate-zone early-spring feeders, particularly in the family Lasiocampidae. These species emerge from diapause early in spring, when temperatures are low, to take advantages of high quality food and a relatively enemy-free space (Despland, 2018). They exhibit a suite of traits to counteract the associated low temperatures, including collective thermoregulation, dark coloration and dense setae (see **Table 1**). Another adaptive suite of traits is seen in tropical herding larvae, particularly in the Ithomiini and Romaleidae (Despland, 2020; Renteria et al.): a phenotype including gregariousness and feeding on toxic plants, which provides larvae with both competitor-free space and with the potential for sequestering toxins for their own defense. Another potential trait that would warrant further attention in these species is social immunity: does feeding on toxic plants protect larvae against pathogens (pharmacophagy) and help counter the higher disease risk associated with group-living (Costa, 2018)? Finally, this trait combination appears particularly frequent in early-succession or disturbed tropical habitats rather than in primary forest (Rathcke and Poole, 1975; Young and Moffett, 1979; Inouye and Johnson, 2005; Despland and Santacruz-Endara, 2016; Despland, 2020); and this habitat association would warrant further investigation.

Some gregarious species that feed on toxic plants are also brightly colored. Indeed, grouping, feeding on toxic plants and aposematic coloration together form a suite of traits that harnesses the advantages of toxic plants to avoid predation. Grouping and aposematism are frequently associated (Ruxton et al., 2019), and one potential evolutionary scenario, first proposed in locusts (Acrididae), suggests that insects feeding on toxic plants acquire warning color when bottom-up driven grouping makes crypsis impossible (Sword, 1999; Despland, 2005). Thus, the brightly colored nymphs of a Romaleid grasshopper are thought to remain in a herd to overcome plant defenses (see **Table 2**), and it's been suggested that this grouping facilitates the evolution of aposematism (Despland, 2020). A similar process could explain the behavior of diprionid sawflies: the chemically-defended larvae aggregate to overcome leaf toughness or to thermoregulate (Ghent, 1960; Fletcher, 2009) and adopt stereotyped behaviors including regurgitation and head-waving to warn predators that they are unprofitable prey (Codella and Raffa, 1993). Synergies between bottom-up and top-down pressures can thus help explain the evolution of herding in species where multiple benefits are observed (Fletcher, 2009). According to this scenario, cooperation that first evolved as an adaptation to overcome bottom-up plant defenses can also form part of an aposematic defensive phenotype that protects from top-down forces.

CONCLUSION

Among the evolutionary drivers underlying larval herding behavior, collective thermoregulation is reasonably well-established. However, although pressures from host plants and predators/parasitoids are often cited as important, they have received less critical analysis. In particular, further work on escaping induced plant defenses and on the relationship between grouping and warning signals could open up important new perspectives in the fields of plant-insect interactions and aposematic theory respectively.

It remains far from clear how collective feeding would allow larval insects to overcome or circumvent plant chemical defenses (see **Table 2**). One suggested mechanism is that insects feeding in synchrony maximize food intake before induced defenses become expressed (Anderson et al., 2015). The study of plant metabolic pathways underlying induced defense is a field that is progressing rapidly, which provides opportunities for investigating benefits to collective feeding at the molecular level [for example de Bobadilla et al. (2021)]. Improved understanding of these advantages could generate meaningful insights into the temporal and spatial feeding patterns of herbivores in general.

Similarly, many questions remain about how aposematism first evolved and how the costs and benefits of warning coloration depend on context (Ruxton et al., 2019). It is increasingly apparent that the adaptive value of color defenses must be understood in the context of suites of functionally related traits that tend to co-vary (Caro and Ruxton, 2019). Indeed, an organism's overall phenotype combines multiple traits and is a response to multiple selection pressures (Pigliucci, 2003). For instance, feeding on toxic plants, gregarious behavior and warning coloration are traits that are frequently expressed together, and that can also be associated with sluggishness, slow growth rate and conspicuous positioning (Despland, 2020). Physiological and biochemical traits related to detoxification, transformation and/or sequestration of plant compounds are likely also associated. Phenotypic integration (Pigliucci, 2003) implies that the adaptive value of each of these traits must be explored in the context of variation in the other functionally related traits. Investigating interactions between these traits and how they are shaped by both bottom-up and top-down selection pressures could provide novel insights to the field of aposematic theory. More generally, applying the phenotypic integration approach to studying the different suites of traits that include larval herding (e.g., the thermoregulating early-spring feeders, the aposematic toxic-plant eaters, etc.) could provide a useful framework to make sense of the complex diversity of social behaviors of herbivorous insect larvae.

In conclusion, this paper shows abundant evidence for direct benefits to larval herding, suggesting that kin selection is not required to explain why hatching insect siblings aggregate. It is worth mentioning that this does not shed any light on the question of whether kin selection occurs as well, since kin selection and natural selection can operate as independent processes (West et al., 2021). Theoretical models of the evolution of insect sociality also examine the potential roles of various levels of selection, raising the possibility that selection could operate on the whole group as well as on the individual (Traulsen and Nowak, 2006; Hölldobler and Wilson, 2008). Indeed, interactions between group members can generate emergent group-level traits that influence individual survival (Wellington, 1960; Myers, 2000): for instance in *E. lanestris,* maintenance of an intact tent is the best predictor of survival of at least one individual of a group (Ruf and Fiedler, 2005). Larval herds thus provide a model system amenable to examining group-level selection, one that is perhaps particularly tractable due to its simple demographic structure.

Finally, it must be noted that only a small minority of herding larvae have been studied and therefore it would be premature to generalize about selective drivers of this alternative yet surprisingly widespread form of insect sociality. Tropical species in particular exhibit a wide range of striking collective behaviors that remain uninvestigated, and for which we can at present only speculate as to their function: for example, rolling swarm caterpillars[1] or wriggling bunches of sawfly larvae[2]. The temperate zone bias (Zuk, 2016) applies to the study of cooperative behavior in insect larvae as well as to other areas of ecology, and implies that there remains much to be discovered.

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[1] https://www.wired.com/2013/07/why-are-these-caterpillars-climbing-over-each-other-the-surprising-science-behind-the-swarm/

[2] http://www.storytrender.com/24762/social-sawflies-band-together-strange-defense-mechanism/

AUTHOR CONTRIBUTIONS

ED wrote the manuscript.

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The Architecture of Cooperation Among Non-kin: Coalitions to Move Up in Nature's Housing Market

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The evolution of cooperation among non-kin poses a major theoretical puzzle: why should natural selection favor individuals who help unrelated conspecifics at a cost to themselves? The relevance of architecture to this question has rarely been considered. Here I report cooperation among non-kin in social hermit crabs (*Coenobita compressus*), where unrelated conspecifics work together to evict larger individuals from a housing market of architecturally remodeled shells. I present (1) the first detailed description of natural coalitions in the wild and (2) a theoretical framework, which examines the evolutionary benefits to each coalition member and predicts when forming a coalition will be successful. In the wild, important ecological and social constraints exist, which are built into the model. Based on these constraints, I show that coalitions can be a successful strategy if several key criteria hold: the coalition is necessary, effective, stable dyadically, and stable polyadically. Notably, the "splitting the spoils" problem—which often undermines non-kin cooperation—is eliminated via architecture: a small individual (C) who helps a medium individual (B) to evict a large individual (A) will ultimately benefit, since C will get B's left behind shell after B moves into A's shell. Coalitions, however, can break down due to added layers of social complexity involving third-party "free riders" and "cheaters," which strategically butt in the architectural queue and thereby steal incentives from the smaller coalition member. Overall, therefore, substantial scope exists for both cooperation and conflict within nature's housing market of architecture. Experiments are now needed to directly test the impact on coalitions of architecture, from the interior of homes up to whole housing markets.

Keywords: architecture, coalitions, cooperation, conflict, cheaters, free riders, housing market, sociality

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> "But it will be like an old abandoned shell. There is nothing sad about old shells."
> –The Little Prince, p. 91

INTRODUCTION

Cooperation poses one of the major theoretical puzzles in evolutionary biology. Why should natural selection favor individuals who help others at a cost to themselves? Fifty years of theoretical and empirical work has demonstrated the importance of shared genetic material in promoting cooperation among kin: kin share genes in common, and these genes can be indirectly passed on through a genetic relative, so helping kin can be evolutionarily favored (Hamilton, 1964a,b).

An elegant theoretical distillation for kin selection and inclusive fitness is Hamilton's rule ($rB > C$), which has been supported by an abundance of empirical studies on cooperation among kin (reviewed in Bourke, 2011). Yet explaining and predicting cooperation among non-kin has remained a central theoretical challenge ever since Darwin (Rubenstein and Abbot, 2017). In contrast to kin, non-kin have no vested genetic interest in helping one another, so why should non-kin ever cooperate?

Analogous to the alignment of genetic interests among kin, there may exist other significant, if more ephemeral, alignments of evolutionary interests among non-kin, which can potentially favor the evolution of cooperation among non-kin (Hammerstein, 2003; Akçay, 2018). Several theoretical solutions have been proposed for non-kin cooperation (Mesterton-Gibbons and Dugatkin, 1992), including reciprocal altruism (Trivers, 1971; Axelrod, 1984), indirect reciprocity (Nowak, 2006; Nowak and Highfield, 2012), group selection (Sober and Wilson, 1999), as well as various types of mutualisms, in which cooperating parties need not act altruistically but can instead mutually benefit simultaneously (Clutton-Brock, 2009). Coalitions represent a form of non-kin cooperation which cuts across many theoretical explanations and which may be mediated by a diverse array of ecological and evolutionary variables (Harcourt and de Waal, 1992; Mesterton-Gibbons et al., 2011). In a coalition, two or more unrelated individuals cooperate, opportunistically working together as a team, often to achieve a cooperative goal that no one individual could attain alone (Sigmund, 1993). Interestingly, the members of a coalition need not have interacted before, and their cooperation can be relatively temporary, arising only because their selfish evolutionary interests momentarily align (Sigmund, 2010). Much theoretical modeling (e.g., Mesterton-Gibbons et al., 2011) has therefore been devoted to understanding how and why the evolutionary interests of coalition members can overlap, including when this synergy may break down, thereby unraveling the cooperation. Notably, even if a temporary alignment of interests enables a coalition to attain its cooperative goal, a major dilemma exists once this goal is successfully realized: splitting the spoils that are the rewards of the joint cooperative effort (Harcourt and de Waal, 1992). This so-called "splitting the spoils" problem often arises because one member of the coalition, particularly the more powerful one, may monopolize the resulting spoils, thereby eliminating any evolutionary incentive for the other, less powerful coalition member to have even cooperated at all. Consequently, a fundamental mystery must be resolved for all coalitions: not just how and why the evolutionary interests of non-kin align to commence cooperation, but also how and why such non-kin cooperation perseveres and is not ultimately undermined by the "splitting the spoils" problem. Insight into potential solutions to this mystery might be had by considering coalitions in the context of architecture.

Architecture permeates the lives of many organisms—from microbes to invertebrates to vertebrates to humans—and it is represented by both the size as well as the internal and external design of an immense variety of constructed and modified homes and dwellings, such as burrows, nests, and other built structures in which animals live (Laidre, 2021a). Such architecture can thus provide an evolutionary setting (Akçay, 2020) in which social behaviors and cooperation may evolve. Yet despite half a century of empirical study of animal architecture (von Frisch, 1974), as well as an equally long historical span of theoretical models of coalitions (Mesterton-Gibbons et al., 2011), these two fields of inquiry have largely existed independently. To my knowledge, no studies—theoretical or empirical—have considered the intersection of architecture and coalitions. Critically, architecture channels and constrains individuals' physical movements (von Frisch, 1974), social decisions (Pinter-Wollman et al., 2017), and overall living parameters (Gould and Gould, 2007; Arndt and Tautz, 2013), with architectural dwellings also often being limited in their availability and especially valuable as resources (Hansell, 2005). Hence, it is plausible that architecture—simply by limiting and constraining individuals' options—might indirectly align the evolutionary interests of unrelated, even competing individuals. Indeed, variable architecture exists precisely because there are often discrete boundaries as to which architectural dwellings are optimal for different individuals (e.g., due to variation in individuals' sizes or other individual-based needs: Bonner, 2006; Arnott and Elwood, 2008; Perna and Theraulaz, 2017). Notably, once different individuals' dwelling requirements vary, the problem afflicting coalitions—that of "splitting the spoils"—might be eased or even entirely eliminated due simply to the fact that each member of a coalition has different architectural preferences for the spoils. Thus, investigating the intersection of architecture and coalitions could yield novel, unexplored insights into theoretical solutions for the evolution of cooperation among non-kin.

As a step toward unearthing deeper connections between architecture and non-kin cooperation, it may be helpful to seek inspiration from an empirical system, one in which architecture is central to individuals' social decisions and ultimately reproductive fitness. With a vast distribution of exchangeable and tradeable homes, the shells of hermit crabs have been referred to as "nature's housing market" (Vermeij, 1993, 2010; Laidre, 2012a; Laidre and Vermeij, 2012). This housing market represents an architectural platform that can generate rich scope not only for conflict, but also potentially for cooperation (Scales, 2015). In particular, highly social terrestrial hermit crabs (*Coenobita* spp.) are dependent upon conspecifics for architecturally remodeled shells, which have carved out interiors, and these shells can only be acquired after fellow conspecifics die or are evicted (Laidre, 2012b, 2018a, 2021b; Valdes and Laidre, 2019; Doherty and Laidre, 2020). Furthermore, due to a planktonic dispersal stage in the ocean, which separates kin early in their lives before they reach land, these social hermit crabs interact almost exclusively with non-kin (Laidre, 2014). Each individual crab interacts and competes with conspecifics, while attempting to "move up" to larger shells in the housing market, thereby advancing its reproductive success (Laidre, 2010, 2013a,b; Steele and Laidre, 2019). Interestingly, despite their selfish individualistic pursuits, social hermit crabs have frequently been observed cooperating among non-kin by forming temporary coalitions, in which two individuals work together to evict another individual from its shell (e.g., Bates and Laidre, 2018; Laidre, 2018b; Doherty and Laidre, 2020). However, there has been no in-depth theoretical consideration of how and why these coalitions emerge; nor has there been any analysis of what prevents these coalitions from being undermined by the "splitting the spoils" problem. By examining the architectural housing market of these coalitions, including the social processes by which architectural vacancies higher in the housing market flow down to others below (Laidre, 2019a), it may help shed light on how and why cooperation among non-kin arises in such a fiercely competitive housing market.

Here I leverage over a decade of naturalistic observations of coalitions among the social hermit crab species *Coenobita compressus* to build a theoretical framework, which seeks to elucidate how and why coalitions are favored within the architectural context of housing markets. Like the theoretical framework originally developed to understand baboon coalitions (Noë, 1990, 1994), which has since been generalized more broadly (Noë et al., 2001), the theoretical framework developed here on coalitions among social hermit crabs might ultimately be extended to other systems with architecture, spurring greater empirical study and experimental tests. I begin by detailing the behaviors and players that underpin coalitions among social hermit crabs, including: the two members comprising the coalition; the target that the coalition seeks to evict; each party's decision options; and the relevance of outside third parties from the broader collective of "fission-fusion" social groupings (Couzin and Laidre, 2009). Next, I leverage these empirical details as a foundation for the theoretical framework, which distils down relevant ecological, evolutionary, and social variables, all of which interact to dictate the benefits and costs accruing to each party. This theoretical framework not only seeks to illuminate why coalitions exist in social hermit crabs, it also seeks to reveal more broadly why architecture and housing markets can foster coalitions. Critically, the theoretical framework generates unique, testable predictions about which specific dyads will versus will not form coalitions, which coalitions will be stable, and when dyadically stable coalitions may nevertheless break down due to polyadic social complexity involving third-party cheaters and free riders. Ultimately, all the predictions from this theoretical framework can be subject to future tests (see Discussion), including experimental tests within social hermit crabs, as well as within other systems where coalitions intersect with architecture.

EMPIRICAL FOUNDATION: NATURAL COALITIONS IN THE WILD

Coalitions among social hermit crabs (*Coenobita compressus*) have been observed for over a decade by the author, his collaborators, and his students, having been referred to in prior studies of many other aspects of this study system (see Laidre and co-author references spanning 2010 to 2021 in our study population in Osa Peninsula, Costa Rica). Yet systematic data has not been collected on these coalitions, and aside from relatively brief mentions—e.g., "Intriguingly, unrelated individuals can also team up in opportunistic coalitions, jointly evicting third parties from their shells" (Laidre, 2018b, p. 239)—no more detailed descriptive accounts exist in the literature. Here I therefore give a more in-depth description of these natural coalitions in the wild, including the first published photographs (**Figures 1**, **2**). These descriptions provide an empirical foundation for the subsequent main focus of the paper, namely the theoretical framework that follows.

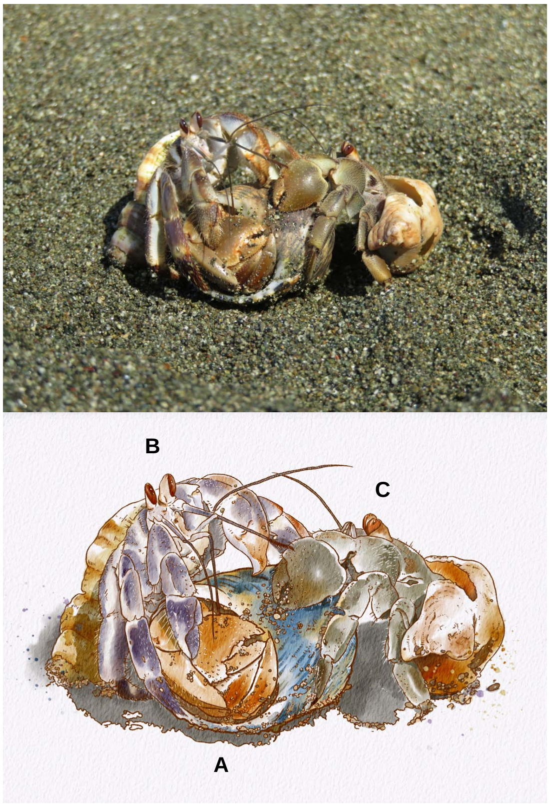


FIGURE 1 | Natural coalition in the wild among social hermit crabs (*Coenobita compressus*). A two-member coalition seeks to evict a bigger individual from its shell. The coalition involves a smaller-sized individual (labeled C) alongside a medium-sized individual (labeled B), both of whom are teaming up and jointly working together to pull out a larger-sized individual (labeled A), who is flipped on its back. Photo by Mark Laidre (taken in 2017 in Osa Peninsula, Costa Rica). Based on the above picture, an artist's illustration (courtesy of Bella Li) is shown below of the same coalition, with individuals labeled as noted.

Coalitions typically involve two individuals (a pair), with a third individual being the target that the two-member coalition seeks to evict from its shell (**Figure 1**). Both members of the coalition have shells of their own, but these individuals and their shells are virtually always smaller than that of the target individual and its shell. Sometimes, based on the commotion and struggle generated during an attempted eviction, additional individuals—beyond the target and the core two-member coalition—are attracted to the area. These additional individuals—referred to as "third parties" or "bystanders"—are not part of the actual coalition, since they do not help at all to evict the target. Generally, third parties simply wait in the vicinity and sometimes position themselves in a social chain, which emanates from the back of the shell of one or both of the coalition members (**Figure 2**). This positioning in a social chain enables third parties

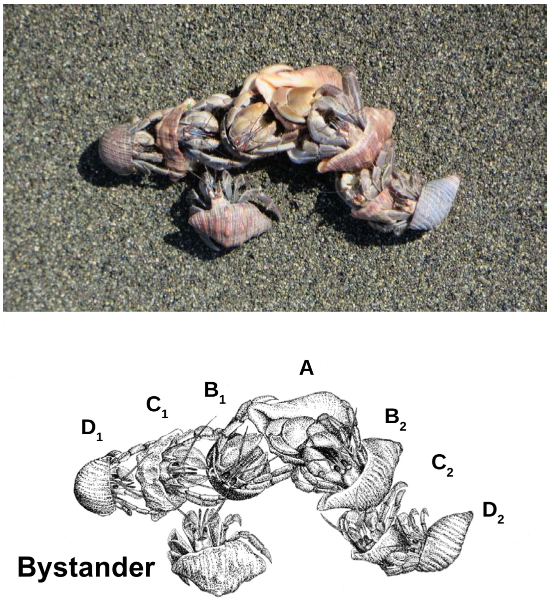


FIGURE 2 | More socially complex, natural coalition in the wild among social hermit crabs (*Coenobita compressus*). The polyadic arrangement involves a core coalition of two individuals (labeled B_1 and B_2) who seek to evict a bigger individual (labeled A). The triad (A, B_1, and B_2) is surrounded by third parties (labeled C_1, C_2, D_1, D_2, and Bystander). B_1 and B_2 each exist at the head a social chain (one chain being B_1, C_1, and D_1, and the other chain being B_2, C_2, and D_2). Notably, these additional individuals emanating from behind B_1 and B_2 provide no help to the core two-member coalition. Rather, these additional individuals are merely "free riders" that may indirectly profit if the coalition succeeds at evicting the target. In some cases, third-party "free riders" may act as "cheaters," stealing the incentives from one of the coalition members by strategically butting in the queue without providing any help. An individual labeled "bystander" simply waits in the vicinity, but is not part of either of the social chains. Photo by Mark Laidre (taken in 2014 in Osa Peninsula, Costa Rica). Based on the above picture, an artist's illustration [from Laidre (2018b)] is shown below of the same coalition, with individuals labeled as noted.

to indirectly benefit, since in the event an eviction succeeds, it can catalyze a succession of back-to-back shell swaps (see Laidre, 2019a). Third parties are thus, in effect, "free riders" (Sigmund, 2010), since their positioning around the coalition offers no advantage whatsoever to the coalition itself as it works to evict the target. Indeed, whether third parties are positioned in a chain or not, they merely wait, performing no pulling actions and never adding any strength or providing any help to the two-member coalition. Interestingly, based on precisely where third parties position themselves, some may potentially even undermine the coalition (see below), effectively acting not merely as "free riders" but as "cheaters" (Sigmund, 2010). Finally, if too many bystanders accumulate, it can lead to chaotic jockeying and repositioning, with the original coalition separating.

Whether with third parties present or not, the two members of the coalition attempt to physically evict the target. The target remains flipped on its back (i.e., with the dorsal side of its shell on the ground) and the opening of the target's shell faces upward, allowing both coalition members to use their claws and legs to grab at and pull the anterior portion of the target's body. As the coalition forcibly pulls, the target attempts to resist by clinging inside its shell. Typically, the two coalition members both pull simultaneously; though at times the two may alternate attempts at pulling, each doing so sequentially as one or the other member briefly rests. Both members of a coalition appear strongly involved, in terms of time and effort. Yet coalitions are not always successful. In some cases, one or both coalition members may give up; or the target individual may manage to flip itself over, escape from being pinned down, and run away. If a coalition is successful at evicting the target, the time till eviction occurs can vary widely, from just minutes up to hours (Laidre, personal observation). Once a coalition is successful and the target individual is evicted from its shell, then the evictee is pushed to the side and remains naked and shell-less as one of the coalition members moves into its now empty shell.

To date, the author has observed no evidence for "advance planning" (Shettleworth, 2010) or "complex coordinated signaling" (Laidre and Johnstone, 2013) between the two members of a social hermit crab coalition. In particular, the two coalition members do not travel side-by-side and they do not simultaneously choose who to target for eviction. Instead, these coalitions appear to originate through a simple series of step-by-step individual decisions, in which two, otherwise independent and unrelated individuals opportunistically converge on an acceptable target. Indeed, in all the hundreds of instances of natural coalitions that the author has observed in the wild, an initial lone individual first flips and pins down a target individual, an act which appears to require minimal effort compared to actually pulling the target out of its shell; only later, as the initial lone individual is holding the target individual down and pulling at its body, does another lone individual approach the eviction site and assess the situation. This second individual then can either move on; can choose to do nothing and remain a bystander; or else can join the initial individual in the attempted eviction, thereby forming a coalition. Further experiments on these coalitions (see Discussion) may reveal additional complexity; but presently, simple cues and decision rules (Shettleworth, 2010) appear to underlie the behavioral dynamics of these coalitions. Below I therefore keep the theoretical framework as simple as possible.

THEORETICAL FRAMEWORK: A CONCEPTUAL MODEL FOR THE ARCHITECTURE OF COALITIONS

To begin, I outline a series of basic assumptions, which lay the foundation of the theoretical framework. Each assumption derives from established knowledge about relevant ecological, evolutionary, and social constraints in the system, as well as key aspects of the above natural history description from wild coalitions. The theoretical framework thus remains faithful to important, real world details of the study system, while at the same time potentially being generalizable to other systems. Next, I delineate several possible evolutionary strategies, which

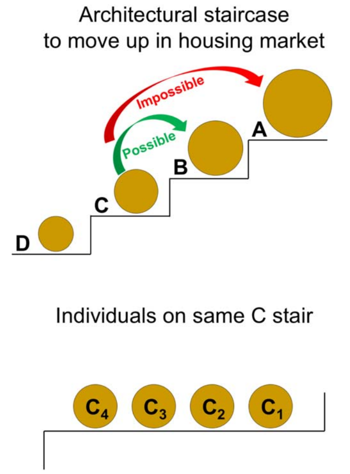


FIGURE 3 | Conceptual model for the architecture of coalitions. In this simple theoretical framework, individuals must climb an architectural staircase to move up in the housing market of shells. The size of each circle in the diagram represents the relative size of individuals and their shells. Letters denote individuals on each separate stair of the staircase, which occupy a given size of shell (i.e., $D < C < B < A$ represents individuals on four separate stairs, each of whom occupy shells of increasing size). Letters with subscripts denote individuals on the same stair (i.e., C_4, C_3, C_2, C_1 represents four distinct individuals on stair C, each of whom occupy shells of equivalent size). For a focal individual on stair C, the green arrow displays a possible move of one step up, while the red arrow displays an impossible move that skips steps (see main text for assumptions of the model). In all subsequent figures, a similar schematic is used to show the model's key predictions about criteria that must hold for a coalition to form and be successful as an evolutionary strategy.

individuals could use to advance in the housing market, including a coalition strategy. Based on the assumptions and available strategies, the theoretical framework then predicts criteria that are critical for coalitions to form and be a successful strategy.

Assumptions

Consider a staircase of architecture (**Figure 3**), each stair of which represents individuals occupying a given size of shell. For every "step up" to a higher stair, individuals on that higher stair occupy a larger shell size than those on the stair below. Letters are used to denote individuals on each separate stair of the staircase (i.e., $D < C < B < A$ represents individuals on four separate stairs, each of whom occupy shells of increasing size). Letters with subscripts are used to denote individuals on the same stair (i.e., C_4, C_3, C_2, C_1 represents four distinct individuals on stair C, each of whom occupy shells of equivalent size). Four key assumptions are made as individuals navigate this staircase:

(*i*) All individuals seek to "move up" the staircase, for it is only by rising to larger shells within the housing market that individuals can increase their reproductive success (Laidre, 2010, 2011, 2013a,b, 2019a,b,c; Steele and Laidre, 2019). Larger shells are vital to reproductive success for both sexes, since they enable females to carry more eggs within their shells and enable males to grow bigger, which is essential to gaining access to mates. In our study population, shells span an order of magnitude, from 5 mm in shell diameter (inhabited by the very smallest individuals) to over 50 mm in shell diameter (Laidre, 2012a).

(*ii*) Problematically, every available shell is occupied across all stairs of the staircase (Laidre, 2010, 2012b; Doherty and Laidre, 2020).

(*iii*) Furthermore, individuals on stairs above are more powerful than those on stairs below, because a tight correlation exists between larger shells being occupied by bigger bodied individuals (Abrams, 1978; Laidre, 2014; Valdes and Laidre, 2018, 2019). Individual condition may occasionally fluctuate (Roberts and Laidre, 2019), but other than molting (when individuals become highly vulnerable) such fluctuations may be slight. Therefore, bigger individuals (who occupy stairs above) are stronger than smaller individuals (who occupy stairs below).

(*iv*) Finally, individuals can only advance one stair at a time: they cannot "skip steps" when moving up the staircase. For example, an individual on stair C cannot leap directly up to stair A, without first moving to stair B (**Figure 3**). This architectural constraint exists because too large a shell—relative to an individual's current body size—is sub-optimal (Laidre and Trinh, 2014). Indeed, for a small bodied individual to carry the weight of too large a shell, is energetically costly and inhibits its growth (Osorno et al., 2005). Furthermore, a small bodied individual is incapable of completely filling and therefore holding onto too large a shell, making it easily evictable and powerless to defend that shell (Laidre, 2021c). Hence, individuals prefer shells of the appropriate, next size up relative to their current body size. Individuals thus must advance in a step-wise manner, which requires discrete moves to each subsequent stair in the staircase.

Possible Evolutionary Strategies

The above assumptions imply that the only way for individuals on lower stairs to move up is when individuals on higher stairs above them vacate their shells, which only occurs if those individual either die or are evicted. Three evolutionary strategies are therefore available for individuals on lower stairs:

- "Wait": wait till those above you either die or are evicted by individuals other than yourself.
- "Alone": alone try to evict another individual above you.
- "Coalition": in a coalition with a conspecific, jointly try to evict another individual above you.

Individuals may pursue a mixed strategy, involving all three of the above strategies. Yet the basic division into these three strategies is justified, given that individuals can be observed performing each one in nature (Laidre, 2014). Indeed, the "Alone" strategy cannot simply be interpreted as a precursor or gamble on cooperation: single individuals attempt evictions in isolated areas, with a low-level of conspecific traffic and with visual barriers, which appear to preclude another conspecific from joining them in a coalition. Furthermore, in some instances where an individual is attempting an eviction all by itself, but then is later joined by a conspecific, it will kick this attempted joiner back, reinforcing that "Alone" is indeed its own distinct strategy.

Importantly, the first two strategies ("Wait" and "Alone") may not be successful in the absence of the third strategy ("Coalition"). With respect to the "Wait" strategy, death via predation is rare in this system, since architecturally remodeled shells remain outside the bite force of most predators on land (Laidre et al., 2012). Instances of conspecific death therefore occur almost exclusively due to conspecific-induced evictions (Valdes and Laidre, 2019). Moreover, aside from such eviction events, the time individuals would need to wait till others above them died naturally would mean those who waited would never have a chance to grow big enough to reproduce (Laidre, 2018a). Similarly, with respect to the "Alone" strategy, an individual on a lower stair may not, by itself, be able to easily overcome an individual on a higher stair: evicting such a larger individual from its larger shell may be challenging for a smaller individual (although cases may arise where it becomes possible due to the larger individual's condition being severely compromised, e.g., Osorno et al., 1998, particularly if the larger individual is vulnerable due to molting). Thus, if individuals on lower stairs cannot simply wait and often cannot go at it alone, then the "Coalition" strategy represents a vital means of moving up. Below I ask when coalitions will form and be successful. For illustrative purposes, I focus on a coalition involving B and C, which work together to evict a target A (see **Figure 1**).

Predictions

For a coalition to form and be successful as an evolutionary strategy, the theoretical framework makes a series of predictions about key criteria that must hold. These predictions follow logically from the assumptions and available alternative strategies laid about above. In particular, for a coalition to form and be successful it must be *necessary*, *effective*, *stable dyadically*, and *stable polyadically*. Below I elaborate on each of these criteria.

Necessary

Coalitions must be necessary (**Figure 4**), otherwise individuals would by default be better off pursing either the "Wait" or "Alone" strategies. As noted above, the "Wait" strategy will be futile when natural death of individuals on stairs above is rare. Likewise, the "Alone" strategy will be unsuccessful whenever B is unable to evict A by itself. Hence, an individual B should only pursue the "Coalition" strategy (i.e., initiate an eviction attempt where fellow conspecifics can easily join in) if it is necessary, meaning that, without help, B would not be powerful enough to overcome A's resistance:

$$\text{Power (B)} < \text{Resistance (A)}.$$

Multiple variables, in addition to raw body size, may potentially contribute to the realized levels of power and resistance. Yet if the above is true, then it follows, given the correlation between shell size and body size in assumption (*iii*), that an individual on an even lower stair (C) should be even less capable of evicting A by itself:

$$\text{Power (C)} << \text{Resistance (A)}.$$

Effective

If a coalition is necessary, then it must also be effective (**Figure 5**) at overcoming the target for it to be successful. Critically, a coalition can only be effective when the combined power of both coalition members is sufficient to exceed the target's resistance and thereby pull the target out of its shell. The strength of different coalition members might combine additively or in more complex non-linear ways. Regardless, a smaller coalition member (C) must be strong enough to contribute sufficient additional power, such that the combined power of the coalition (B and C) is effective at exceeding A's resistance:

$$\text{Power (B + C)} > \text{Resistance (A)}.$$

In contrast, other coalitions (e.g., B and D), in which the smaller of the two coalition members (D) is too weak, may prove ineffective:

$$\text{Power (B + D)} < \text{Resistance (A)}.$$

Stable Dyadically

Coalition Members Should Have Aligned, Not Competing Evolutionary Interests

Even if a coalition is necessary and effective, its success might be undermined if it is destabilized dyadically, due to

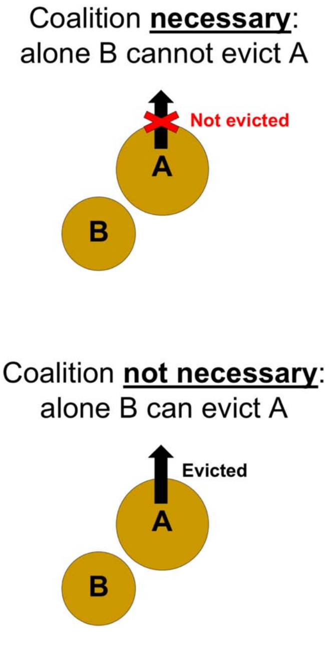


FIGURE 4 | For a coalition to form and be successful, it must be *necessary*. In this case, alone B cannot evict A.

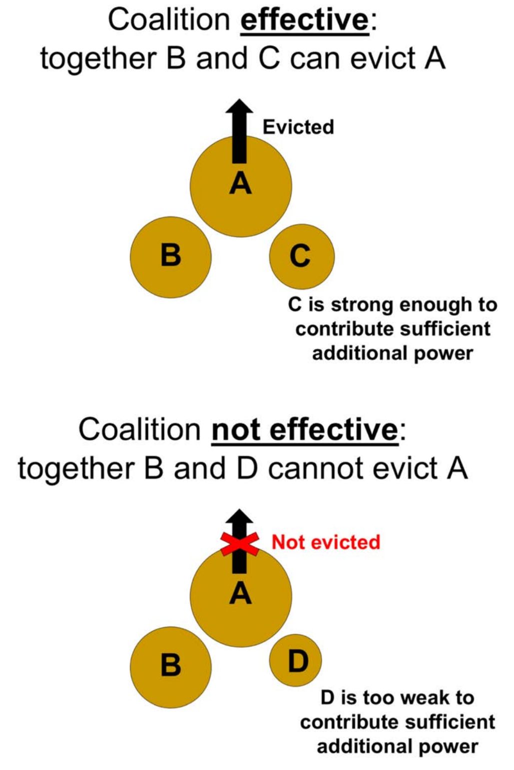


FIGURE 5 | For a coalition to form and be successful, it must be *effective*. In this case, together B and C can evict A.

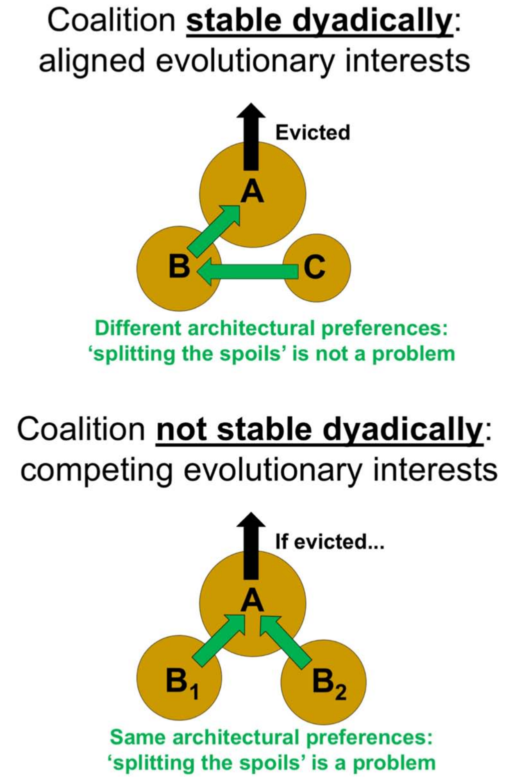


FIGURE 6 | For a coalition to form and be successful, it must be *stable dyadically*. In this case, B and C have aligned evolutionary interests. This alignment exists due to their different architectural preferences. Thus, after A is evicted, B moves into A's shell and C moves into B's left behind shell, thereby resolving the "splitting the spoils" problem. In contrast, a coalition will not be stable dyadically if individuals have the same architectural preferences, since then the coalition members will have competing evolutionary interests: if A were to be evicted, both B_1 and B_2 would compete over moving into A's shell, thereby generating a "splitting the spoils" problem.

competing interests between the two members of the coalition. It is therefore important that a coalition be stable dyadically (**Figure 6**) by avoiding conflict and retaining an alignment of interests, from the time the coalition first starts working together to evict the target up to even after the eviction has been realized. Given that post-eviction only one member of the coalition can acquire the shell of the evictee, how do both members of the coalition benefit evolutionarily? In other words, what resolves the "splitting the spoils" problem for this single emptied shell?

Notably, as a corollary of the architectural constraint in assumption (*iv*), it follows that variable architectural preferences exist for members of a coalition that occupy different stairs. For example, an individual on stair B will seek stair A as its optimal next step, while an individual on stair C will seek stair B as its optimal next step. As a consequence of these divergent preferences, an optimal outcome can arise naturally in which both members of the coalition benefit (**Figure 6**): once the target A is evicted, then B can realize its preferred move into A's empty shell, and after that C can realize its preferred move into B's left behind shell. In effect, C helps B move into A's shell. And such helping is in C's selfish interest, because subsequently C receives B's passed down shell. Architectural constraints, with their associated variation in individuals' architectural preferences and resource values, can thus eliminate the "splitting the spoils" problem.

In contrast, some potential coalitions, despite being both necessary and effective, may be unstable dyadically (**Figure 6**). For example, two individuals that both occupy stair B (B_1 and B_2) could have a combined power that is more than sufficient to overcome an eviction target (A). However, if both individuals were to work together to evict A, then once A is actually evicted, B_1 and B_2 will come into direct conflict: their previously aligned interests will collapse and they will become competitors for A's empty shell. Such a free-for-all would not just entail a 50-50 chance of the spoils, but rather could lead to quite dangerous and costly outcomes for one or both parties. In particular, if two individuals lack dyadic stability, and hence are willing to squabble over an evictee's shell after it is evicted, then there is a non-negligible chance that one or both may ultimately lose their original shells. As these two former coalition partners simultaneously attempt to enter the larger empty shell of the evictee, struggling with one another to be first, then the evictee, as well as other individuals passing by, could easily move into their original shells, potentially leaving them with an even less suitable shell due to their own conflict over the spoils. Perfect harmony and alignment of interests may not always be possible between coalition members. Yet an inherent dyadic instability will exist for those coalitions in which neither member stands to benefit from the shell left behind by the other member (and hence where both members' architectural preference is for the shell of the evictee). Such dyadically unstable coalitions should therefore be less likely to persevere and may also form less frequently than coalitions that are dyadically stable.

Stable Polyadically

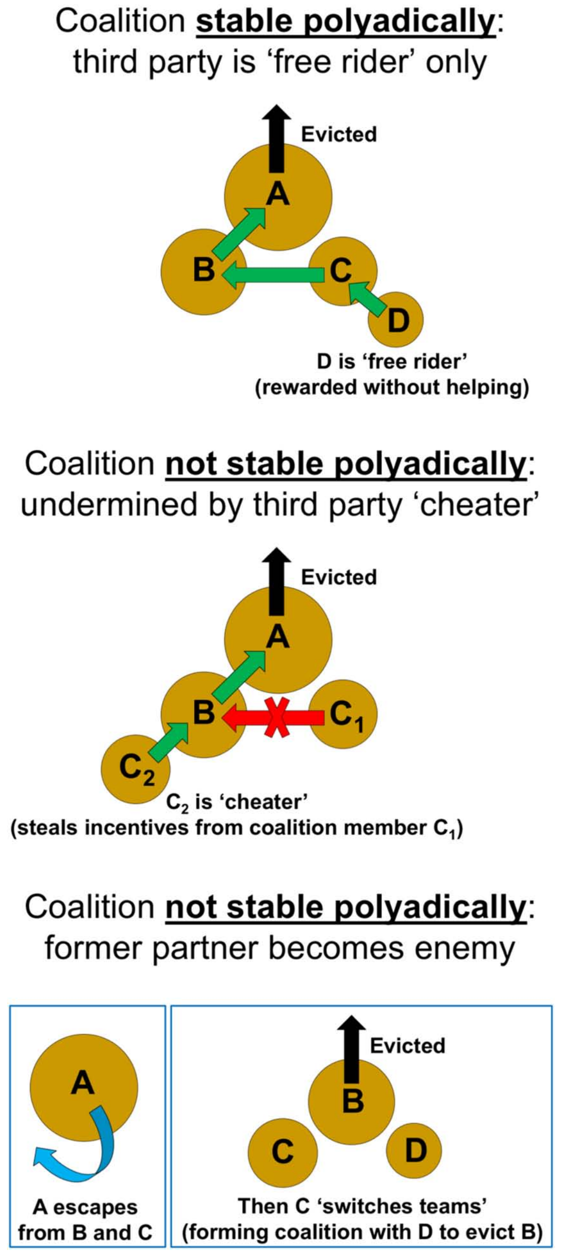
Incentives for Smaller Coalition Member Must Not Be Stolen by Third Party "Cheater"

Even if necessary, effective, and stable dyadically, a coalition poised for success might be destabilized polyadically (**Figure 7**) due to the arrival of third parties. One way such polyadic instability could be introduced is if, in addition to the eviction target (A) and the two core coalition members (B and C), a third party arrives that is on the same stair as the smaller of the two coalition members (C). To differentiate these individuals occupying the same stair, we designate the original coalition member as C_1 and the third party as C_2. Importantly, depending on where C_2 positions itself, it has the potential to eliminate any incentive for C_1 to continue helping as part of the original coalition. This is because for the coalition to function, the two coalition members (B and C_1) must each reach into the target A's shell as they pull, which requires their bodies be oriented inward, while their shells are directed outward, typically on opposite sides of the target (see **Figure 1**). Consequently, C_2 can effectively "butt" in the queue by physical positioning itself immediately behind and clinging to the shell of B (**Figure 7**), thereby being in a strategically superior position—compared to C_1—to move into B's left behind shell, should an eviction succeed. Operationally, C_2 can thus act as a "cheater": without helping at all, and yet by taking the prime position, it can stand to steal all the rewards of C_1's cooperative effort. The more third parties that arrive on this same stair (e.g., C_2, C_3, C_4, etc.), the greater the chance that one or more might cheat in this way, thereby taking away the evolutionary incentives for the smaller coalition member to help, and ultimately leading to a breakdown of the original coalition.

This third-party cheater problem can be averted if the third party that arrives is on a stair below C (i.e., D). In that case, individual D cannot act as a "cheater" but merely as a "free rider" (**Figure 7**). This is because D naturally prefers C's left behind shell. Hence, even if D places itself in the optimal position (i.e., immediately behind C), this will not remove any incentives for C to continue helping as part of the original coalition. A separate problem, however, arises in terms of polyadic stability if a third party D arrives (see below).

Smaller Coalition Member Must Not "Switch Teams" and Become Enemy

Another way otherwise necessary, effective, and dyadically stable coalitions might be destabilized polyadically is if the smaller coalition member (C) turns on its partner (B), becoming an enemy (**Figure 7**). This dangerous flip is possible if a third party (D) arrives: for then C has the option of "switching teams" and teaming up with D in a new coalition, one where its former coalition partner (B) now becomes the target of eviction. As more individuals from stair D arrive (e.g., D_1, D_2, D_3, etc.) the likelihood increases that such a coalition switch will



Coalition **stable polyadically**:
third party is 'free rider' only

Coalition **not stable polyadically**:
undermined by third party 'cheater'

Coalition **not stable polyadically**:
former partner becomes enemy

FIGURE 7 | For a coalition to form and be successful, it must be *stable polyadically*. In this case, a third-party "free rider" D will not undermine the coalition. D can benefit, despite not helping at all, since after the eviction succeeds, B and C will both move into their preferred shells, and then D can move into C's left behind shell. In contrast a coalition will not be stable polyadically if a third-party "cheater" (C_2) steals incentives from one of the coalition members (C_1) by placing itself in a superior position to move into B's left behind shell. Furthermore, a coalition will not be stable polyadically if a former coalition partner (C) "switches teams," turning on B to become an enemy, by forming a new coalition with D to evict B. This scenario may arise if the original target (A) escapes, with only the triad (of B, C, and D) remaining.

occur, undermining the original coalition arrangement. Polyadic instability from such upended coalitions will most likely be catalyzed if the original target (A) manages to escape, since then only the triad (of B, C, and D) is left. Yet whether C and D will then unite to form a coalition against B depends recursively on all the criteria outlined above, requiring that this new coalition be necessary, effective, stable dyadically, and stable polyadically.

DISCUSSION

Insights From Conceptual Model

The relevance of architecture for coalitions has rarely been considered, despite architecture permeating the lives of many animals (von Frisch, 1974; Hansell, 2005; Gould and Gould, 2007; Arndt and Tautz, 2013; Perna and Theraulaz, 2017; Pinter-Wollman et al., 2017; Laidre, 2021a), and potentially having profound impacts on social dynamics and cooperation among non-kin. After laying an empirical foundation, based on the natural coalitions within the housing market of social hermit crabs, the main purpose of this paper was to then build a theoretical framework, which could predict when coalitions would form and be successful in an architectural context. The conceptual model predicts several key criteria that must hold if coalitions are to form and be successful: coalitions must be necessary, effective, stable dyadically, and stable polyadically. An overarching prediction of the model is that coalitions will only succeed when the distribution of architecture and power—among the coalition members and the evictee—yields an optimal route up the staircase for both members of the coalition to advance in the housing market. Interestingly, the model highlights a novel architectural solution for the evolution of cooperation among non-kin, by resolving the problem that often undermines coalitions—"splitting the spoils"—based on variation in architectural preferences. This simple model of the architecture of coalitions could easily be modified and extended to other architectural contexts, possibly offering insights in other systems, where the constraints imposed by architecture may likewise align unrelated individuals' interests and ultimately fuel non-kin cooperation.

Are Non-kin Coalitions Involving Architecture Unique to Certain Species?

The coalitions described herein among hermit crabs have been observed and reported only in the highly social terrestrial genus *Coenobita*, not in other hermit crab species that are aquatic (e.g., *Pagurus* spp., Arnott and Elwood, 2007). Indeed, despite over 15 years of observations of these other, less social hermit crabs, neither the author, his students, or his collaborators have ever observed coalitions in any of these less social species (Laidre, 2007, 2009, 2011; Laidre and Elwood, 2008; Laidre and Greggor, 2015; Greggor and Laidre, 2016; Valdes and Laidre, 2018; Doherty and Laidre, 2020). Less social marine hermit crabs readily acquire new shells from sources other than conspecifics (Laidre, 2011; Valdes and Laidre, 2018), which likely nullifies any need for coalitions or non-kin cooperation among conspecifics. Interestingly, less social marine hermit crabs do exhibit inter-specific cooperative mutualisms (Bergstrom et al., 2003), in which other species provide valuable services in return for living on or inside a hermit crab's shell (e.g., anemones attached to a hermit crab shell protect the crab from octopus predation: Ross, 1971). Yet as for coalitions and intra-specific non-kin cooperation, these appear unique to the highly social terrestrial hermit crabs. In addition to coalitions, studies of highly social terrestrial hermit crabs have revealed that they differ in many other ways compared to less social marine hermit crabs (Laidre, 2014). Social hermit crabs exhibit the following traits in their fission-fusion (Couzin and Laidre, 2009) social groupings: a much stronger social attraction to conspecifics, both living (Laidre, 2010, 2013a,b; Steele and Laidre, 2019) and dead (Valdes and Laidre, 2019); an evolutionary loss of threat displays (Doherty and Laidre, 2020); specialized social cognition (Laidre, 2018b), including a nuanced understanding of fine-grained social formations (Bates and Laidre, 2018) and a capacity for problem-solving (Krieger et al., 2020); coordinated behaviors used in the social exchange of shells, which are passed down across generations (Laidre, 2019a); as well as an altered sexual morphology, which reduces the danger of shell theft during copulation (Laidre, 2019b). The greater sociality of these *Coenobita* spp. hermit crabs, compared to the less social marine hermit crabs, is linked to their extreme dependence on conspecifics for architecturally remodeled shells, which can only be acquired after conspecifics are evicted or die, and without which individuals cannot survive (Laidre, 2012b).

Further comparative studies can help resolve the phylogenetic limits of coalitions (Bracken-Grissom et al., 2013), potentially revealing additional hermit crab species that might form coalitions when ecological imperatives arise. Even more broadly, beyond hermit crabs, countless other social invertebrates are premier "animal architects" (Gould and Gould, 2007), which may therefore offer additional opportunities (Elgar, 2015) for exploring how and why architecture influences cooperation. Indeed, many species, from invertebrates to vertebrates, inhabit a wide range of burrows of variable size and shape, with individuals frequently evicting fellow conspecifics from their burrows [reviewed in Laidre (2018a)]. Paralleling the collaborative evictions in shell "housing markets" there could be scope for similar collaborative evictions in closely clustered burrows, where certain burrows may be more optimal for specific individuals, depending on their current state (e.g., size, reproductive status, or the presence of additional kin or non-kin that live with them). Furthermore, like the architecturally remodeled shells of social hermit crabs, many other forms of architecture are costly to excavate or to construct (Hansell, 2005), so there may be incentives for other animals to likewise find ways of circumventing such architectural costs by working together to evict current owners in a collaborative effort. Finally, given strong evidence that architecture has influenced cooperation among close kin (e.g., the fortresses of eusocial insects: Queller and Strassmann, 1998; Perna and Theraulaz, 2017; Varoudis et al., 2018), deeper study of architecture's potential impact on non-kin cooperation could be constructive, including further studies of non-kin cooperative nesting among invertebrates (Haney and Fewell, 2018) as well among vertebrates (Riehl, 2013).

Next Steps: Empirical Tests of Model's key Predictions

The present contribution has generated a number of testable predictions, but these predictions remain solely in the theoretical realm and have yet to be explicitly and rigorously tested empirically. Such empirical tests represent the obvious next step (**Table 1**), and can be accomplished both by quantifying elements of natural coalitions in the wild and, even more powerfully, by conducting experimental tests, which can span field conditions as well as controlled laboratory conditions. Moving forward, social hermit crabs, which served as empirical inspiration, can offer a model empirical system for testing many of the theoretical framework's key predictions, thereby helping unearth how and why architecture and housing markets impact coalitions. Social hermit crabs, in particular, lend themselves well to critical experimental tests, since virtually all the variables of theorized importance (the size of individuals; the size of shells they occupy; the relative strength of each individual; the dyadic and polyadic combinations of individuals; and the presence or absence of additional third parties) can be precisely controlled and manipulated.

Naturalistic Tests Based on Systematic Field Measurements

Years of naturalistic field observations on coalitions among social hermit crabs have been made, but still many basic empirical questions remain, including the frequency with which these coalitions form across "fission-fusion" social groupings (Couzin and Laidre, 2009), which span beach and forest (Steele and Laidre, 2019). An important first empirical step will be collecting systematic field measurements during transects of the frequency, the relative success rate, and the exact composition of natural, spontaneously forming coalitions in the wild. Previously, my students, collaborators, and I have not interrupted ongoing coalitions. However, future studies can take two approaches, one naturalistic and one more invasive, each generating complementary data, which are relevant to testing key predictions of the theoretical framework. First, from a naturalistic approach, coalitions can be followed from their point of initial formation up until their natural end point, which involves either successful eviction or unsuccessful dissolution (i.e., the coalition members separate). At this natural end point, all individuals involved—the two coalition members, the eviction target, and any additional third parties—can be collected, and their shell sizes and bodies can be measured, thereby testing if their relative sizes align as predicted. Second, utilizing a more invasive approach, coalitions can be interrupted in their early stages, while they are still in progress but before their fate has been determined, with all individuals once again being collected. Then these individuals can be tested with a field portable "eviction machine" we recently engineered (Laidre and van Citters, unpublished), which can quantify the biomechanical costs underlying eviction, including both pulling force and resistance force. We can therefore measure the maximum pulling force of the two coalition members (independently and when combined) as well as the maximum resistance force of the eviction target. Altogether, these biomechanical measures, as well as shell size and body measures, can offer a first test of predictions about which distributions of power and architecture yield necessary, effective, and stable coalitions.

Experimental Tests With Socially Engineered Groups

Building on such naturalistic and semi-invasive studies, another step will be conducting experiments with socially engineered groups. Unlike many other coalition-forming animals (e.g., large mammals and primates: Harcourt and de Waal, 1992), which can be challenging to relocate or introduce into new groups, social invertebrates offer exquisite ease in terms of translocating individuals between groups and thereby forming new and artificial group compositions. This line of experimentation with socially engineered groups can be readily accomplished with social hermit crabs. In particular, individuals collected directly from the wild can be assembled into dyads and triads, including those that are predicted to be successful versus not, thereby directly testing how well theoretical predictions match reality in terms of coalition formation and success. For example, which coalitions are necessary and effective can be tested by creating combinations (such as only A and B; only A and C; or A, B, and C), with just the last assembly predicted to lead to A's eviction. Similarly, which coalitions are dyadically stable vs. unstable can also be tested, with B and C predicted to succeed at evicting A, but with B_1 and B_2 predicted to destabilize, given that the members

TABLE 1 | Key open questions about coalitions in social hermit crabs.

- How frequently do coalitions form across variable social, spatial, and temporal dimensions, including different fission-fusion social groupings, different ecological settings spanning beach to forest, and different seasons?
- What is the success rate of coalitions and, if successful, then how long does eviction take?
- What are the relative shell sizes and body sizes of coalition members and their target?
- How powerful are coalition members (independently and together) in their pulling force compared to the resistance strength of their target?
- Which combinations of socially engineered groups are more likely to form successful coalitions?
- In what circumstances does the arrival of third-party cheaters and free riders undermine coalitions?
- Do coalition members to seek privacy during attempted evictions?
- Do eviction targets attempt to attract additional conspecifics to create confusion that destabilizes coalitions?
- How and why does architecture—from the fine-scale architecture within the interior of individual shells up to the large-scale architecture of an entire shell housing market—impact coalition formation and frequency?

To critically test the proposed theoretical framework, future field observations and experiments must answer the above questions.

of the latter pair will prefer and hence compete over the target A's shell. In addition to testing variable social compositions, it will also be possible to explore how coalition formation changes with greater or lesser disparities between individuals, for example by altering what shell sizes specific individuals in the triad initially occupy, and even by impairing specific individuals' strength (e.g., weakening individuals via anesthesia: Osorno et al., 1998). A rich suite of experimental tests using socially engineered groups thus awaits.

Experimental Tests With Third Parties

One key prediction of the conceptual model is that for a coalition to be successful, its dyadic stability (which arises from the alignment of the two coalition members' interests) must not be undermined by polyadic instability (which arises through the arrival of third-party "cheaters" and "free riders"). This prediction—that third parties may imperil a coalition's stability—can be experimentally tested. For example, in the experiments mentioned above with socially engineered groups, a separate chamber door could be used to introduce third parties into the main group's arena, systematically varying the number and size of these third parties, as well as the time at which they are introduced. Likewise in the wild, it is possible to simulate the arrival of third parties via previously utilized experimental techniques, such as tethering live crabs (e.g., Laidre, 2010; Steele and Laidre, 2019); using dynamically moving models of dead conspecifics (e.g., Doherty and Laidre, 2020; see also Laidre and Vehrencamp, 2008); using empty shells that are either jostled (e.g., Laidre, 2013a) or dragged to simulate conspecific movement; or employing arrays of shells as stand-ins for social chains of conspecifics (e.g., Bates and Laidre, 2018). All these methods can test whether subtle changes in the surrounding polyadic social context undermine coalitions. Interestingly, given the potential hazard posed by third parties, it may be in the interests of both coalition members to seek privacy (Strassmann and Queller, 2014) during attempted evictions, not unlike how private locations are sought to evade shell theft during copulation (Laidre, 2019b). Also, from the perspective of the eviction target, the mere possibility that a coalition might be undermined by polyadic social complexity may incentivize the target to attract as many additional conspecifics as possible, in a desperate attempt to destabilize the coalition and create enough confusion for the target itself to escape. Prominent sounds that are produced by social hermit crabs while defending against eviction (Laidre and Symes, 2021) might function in such social eavesdropping, and this acoustic hypothesis can be tested in future playback experiments.

Experimental Tests of Architecture: From Shell Interior up to Whole Housing Markets

In addition to experiments with variable groups and third parties, the very nature of the underlying architecture can provide a source of powerful experimentation. Experimental alteration of architecture can be accomplished from the fine-scale (e.g., the inner architecture within an individual shell) up to the large-scale (e.g., the overall size distribution of an entire shell housing market). Social hermit crabs architecturally remodel the interiors of shells, which is beneficial in terms of lightening the load they must carry on land (Herreid and Full, 1986; Trinh and Laidre, 2016). However, this architectural remodeling has a hidden cost: by eroding out much of the shell's interior, it means there is less for owners to grip upon when resisting eviction (Laidre, 2021c). Thus, in linking architecture to coalitions, one interesting hypothesis is that altering the available grip inside shells could impact the ability of owners to resist eviction, regardless of their baseline strength; and if owners are then more versus less hard to evict, it should in turn change whether coalitions are still necessary or effective. Recently, we have micro-CT scanned natural shells with substantial variation in inner shell grip and have also 3D printed shells with novel architectural variation (Krieger et al., 2020). Future experiments can test how such variation in fine-scale inner architecture impacts many aspects of coalition formation and the frequency with which they occur. Similarly, at the largest architectural scale of the entire shell housing market, the potential exists to alter the overall size distribution of this market, either by introducing or removing set sizes of shells. In theory, such altered size distributions of architecture could dramatically change localized competition levels, profoundly affecting available options for individuals seeking to move up in the housing market. Such architectural changes could also add social pressure on individuals at the top of the housing market, by making it more likely for "revolutionary" coalitions to be incited among those at the bottom or middle of the housing market. Long-term studies that experimentally change the size distribution of housing markets in wild populations thus have the tantalizing potential to test whether these changes also causally influence the frequency of coalitions and which specific coalitions form.

Further Theoretical Work

Finally, alongside these several strands of empirical work, further theoretical work on the intersection of architecture and coalitions could yield broader insights. The main focus herein was developing a relatively simple theoretical framework, which could serve as an initial conceptual model for making testable predictions and driving empirical inquiry. Yet future theoretical work on this topic could build on this foundation by developing more sophisticated formulations—agent-based computer simulations, analytical mathematical models, and game-theoretical models (Maynard Smith, 1982; Sigmund, 1993, 2010)—all of which can explore ramifications of altered ecological and social variables, including in the overall housing market. Coalitions inherently play out in a broader "housing market" as well as "social market" context (Noë et al., 2001; Roth, 2015), in which individuals have many choices for potential coalition partners, as well as for potential targets to evict. Thus, while the predicted criteria for necessary, effective, and stable coalitions appear concrete and straightforward, their computational complexity may be non-trivial due to the broader matrix of potential strategies playing out across an entire market. Coalitions deemed necessary, effective, and stable might nevertheless be ruled out due to alternative decisions by other individuals within the market: for instance, a seemingly necessary, effective, and stable coalition (between small C and medium B to evict large A) might be subverted if smaller individuals D and E team up to evict C. Future theoretical work should account for this complex matrix of strategies by winnowing down all potential coalitions to only those that remain viable in reference to power dynamics and architectural size distributions across the market as a whole. Such theoretical work could also explore how variable levels of conflicting versus aligned interests—in terms of the degree of overlap in architectural preference between two coalition members—shapes the outcome of coalitions, as well as which individuals form coalitions and the overall frequency of coalition formation. Ultimately stronger bridges can be built between theoretical and empirical work on this topic, with empirical data not only testing theory, but inspiring new theory (e.g., computational algorithms of "coalition formation" that integrate information on power and architecture to compute which coalitions function best).

Summary and Future Prospects

The present theoretical framework has sought to address a long-standing question in behavioral ecology and evolutionary biology by proposing a novel solution for the evolution of cooperation among non-kin: architectural constraints. Unlike prior explanations for non-kin cooperation, where "splitting the spoils" can undermine the cooperative relationship, this novel architectural solution predicts that the evolutionary interests of non-kin can coincide, particularly on an architectural staircase with discrete reusable resources, like shells. Coalitions among non-kin can therefore arise even as genetically unrelated individuals all selfishly pursue their own evolutionary interests, competing fiercely over scarce architectural resources. Future work in this area can profitably wed theory with experiment in an interdisciplinary approach spanning biology, engineering, and mathematics to better understand the coalitions that form in nature. The path from original observation to incipient hypotheses to simple conceptual models to experimental tests to full-blown theory is long and winding. Yet ultimately, continued research down this fascinating intellectual path can help build the architectural foundation of a better understanding of cooperative behavior.

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

ML conceived and designed the model, made the original empirical observations that inspired the model, and wrote the manuscript.

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ORIGINAL RESEARCH
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Benefits and Costs of Mixed-Species Aggregations in Harvestmen (Arachnida: Opiliones)

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Many animals form aggregations with individuals of the same species (single-species aggregations, SSA). Less frequently, individuals may also aggregate with individuals of other species (mixed-species aggregations, MSA). Although the benefits and costs of SSA have been intensively studied, the same is not true for MSA. Here, we first review the cases of MSA in harvestmen, an arachnid order in which the records of MSA are more frequent than other arthropod orders. We then propose several benefits and costs of MSA in harvestmen, and contrast them with those of SSA. Second, using field-gathered data we describe gregariousness in seven species of *Prionostemma* harvestmen from Costa Rica. These species form MSA, but individuals are also found solitarily or in SSA. We tested one possible benefit and one possible cost of gregariousness in *Prionostemma* harvestmen. Regarding the benefit, we hypothesized that individuals missing legs would be more exposed to predation than eight-legged individuals and thus they should be found preferentially in aggregations, where they would be more protected from predators. Our data, however, do not support this hypothesis. Regarding the cost, we hypothesized that gregariousness increases the chances of parasitism. We found no support for this hypothesis either because both mite prevalence and infestation intensity did not differ between solitary or aggregated individuals. Additionally, the type of aggregation (SSA or MSA) was not associated with the benefit or the cost we explored. This lack of effect may be explained by the fluid membership of the aggregations, as we found high turnover over time in the number of individuals and species composition of the aggregations. In conclusion, we hope our review and empirical data stimulate further studies on MSA, which remains one of the most elusive forms of group living in animals.

Keywords: alarm signals, aggregation size, autotomy, chemical defenses, dilution effect, ectoparasitism, group living, roosting site

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INTRODUCTION

One of the simplest forms of group living in animals is gregariousness, defined as "the tendency of an animal to aggregate with others such that the animals are in contact with one another, or are nearly so, and that the distribution of the animals in the local environment is extremely patchy" (Vulinec, 1990). Although most theoretical and empirical studies on gregariousness focus on groups formed by individuals of the same species (reviewed in Ward and Webster, 2016), there is increasing evidence that aggregations composed of two or more species are common across the animal kingdom, including birds, mammals, fish, and arthropods (Rasa, 1990; Heymann and Buchanan-Smith, 2000; Zamon, 2003; Boulay et al., 2019; Goodale et al., 2019, 2020). Goodale et al. (2017) recognize two types of mixed-species associations: (a) mixed-species groups, which involve individuals of several species moving together, such as herds of herbivores in the African savanna (e.g., Kiffner et al., 2014), and (b) mixed-species aggregations, which involve individuals gathering around a resource or location, as occurs with many arthropods (reviewed in Boulay et al., 2019). Regardless of the type, the formation of mixed-species associations, especially among predatory species, is remarkable because it requires tolerance and the ability to exchange information with both conspecifics and heterospecifics (**Box 1**).

Research in evolutionary biology aims to understand why animals aggregate by contrasting the fitness benefits of group living with its costs (e.g., Ward and Zahavi, 1973; Parrish and Edelstein-Keshet, 1999; Greenfield, 2015). An overall balance toward benefits can explain the maintenance of gregariousness in a species. Here, we explore the less-studied question of whether the benefits and costs experienced by individuals in single-species aggregations (SSA) also apply to mixed-species aggregations (MSA). To address the benefits and costs of MSA, we first review the cases of MSA in a particular group of animals in which MSA are relatively frequent, the arachnids of the order Opiliones, commonly known as harvestmen. In our review, we compare potential benefits and costs individuals may have by joining MSA or SSA. Then, we present the first empirical investigation on the benefits and costs of MSA in harvestmen. Finally, we use our empirical findings to propose future directions to study group living among heterospecific individuals.

MIXED-SPECIES AGGREGATIONS IN HARVESTMEN: A REVIEW

Arachnids are mostly solitary, but some cases of gregarious, communal, subsocial, and even social species have been described for mites (Saito, 1997), scorpions (Mashberg, 2001), pseudoscorpions (Del-Claro and Tizo-Pedroso, 2009), whip-spiders (Rayor and Taylor, 2006), spiders (Aviles, 1997; Whitehouse and Lubin, 2005; Yip and Rayor, 2014), and harvestmen (Machado and Macías-Ordóñez, 2007). Our review focuses on a particular type of group living, gregariousness. Most cases of gregariousness in arachnids refer to SSA composed of either kin or non-kin individuals (mites: Saito, 1997; spiders: Whitehouse and Lubin, 2005; harvestmen: Machado and Macías-Ordóñez, 2007). However, there are some cases of gregariousness among arachnids in which individuals of two or more species group together. These cases of MSA occur in mites and ticks (e.g., Tsunoda, 2007; Le Goff, 2011), scorpions (Warburg, 2000), spiders (e.g., Hodge and Uetz, 1996; Hodge and Storfer-Isser, 1997), and harvestmen (Machado and Macías-Ordóñez, 2007). Recently, Boulay et al. (2019) reviewed cases of MSA in arthropods, but the main focus of the paper was on insects and only a few examples in arachnids were mentioned. Hence, we aim to expand the topic by providing an in-depth account on the records of MSA in harvestmen.

Harvestman Aggregations

The order Opiliones includes nearly 6,650 species distributed in all continents, except Antarctica (Kury et al., 2020). There are four extant suborders: Cyphophthalmi, Dyspnoi, Eupnoi, and Laniatores. Most of the knowledge about harvestman ecology, behavior, and physiology is concentrated in the latter two suborders (Pinto-da-Rocha et al., 2007), which are also the most diverse, comprising together 90% of all Opiliones (Kury et al., 2020). All cases of gregariousness in harvestmen occur among representatives of Eupnoi and Laniatores (Machado and Macías-Ordóñez, 2007; **Figure 1**). The Eupnoi that form aggregations include exclusively small-bodied, long-legged species that are common in temperate regions, but also occur in tropical forests. In turn, the Laniatores that form aggregations include large-bodied species, with either short or long legs, which occur exclusively in the neotropics.

Aggregations in harvestmen (SSA or MSA) consist of motionless individuals, with their bodies 0–5 cm apart from each other, and their legs usually overlapping or at least in close proximity (Machado et al., 2000). Holmberg et al. (1984) categorized harvestman aggregations into (i) *dense* or *mass* aggregations consisting of hundreds or thousands of individuals packed in high density, facing upward and with their legs hanging down or intertwined (**Figures 1A,B**), and (ii) *loose* aggregations of dozens or a few hundred individuals not densely packed, with bodies oriented in different directions and legs held outstretched or flexed, but never intertwined (**Figures 1C,D**). Both types of aggregations are composed mostly of non-kin subadults and adults in variable sex ratios (Machado and Macías-Ordóñez, 2007; Grether et al., 2014a). Because most harvestman species are nocturnal and sensitive to dehydration (Santos, 2007), aggregations are usually found during daytime and in humid, poorly illuminated places, including under rocks and rotting logs, inside caves, and under dense vegetation (e.g., Juberthie, 1972; Holmberg et al., 1984; Willemart and Gnaspini, 2004; Donaldson and Grether, 2007). The aggregations disperse at dusk, when individuals leave the roosting site to forage and then re-group at dawn (e.g., Machado et al., 2000; Wade et al., 2011; Proud et al., 2012). Finally, harvestman aggregations are more frequent during dry and cold periods, especially fall and winter, and/or in xeric environments (Machado and Macías-Ordóñez, 2007). In temperate regions, some species of Eupnoi form aggregations that remain quiescent inside caves throughout the winter and individuals disperse only at the beginning of spring (e.g., Holmberg et al., 1984; Novak et al., 2004).

Mixed-Species Aggregations

We performed a backward and forward literature search based on the papers contained in Table 11.2 of the chapter ‘Social behavior’ (Machado and Macías-Ordóñez, 2007) of the book *Harvestmen: The Biology of Opiliones* (Pinto-da-Rocha et al., 2007). Using Web

BOX 1 | The challenges of aggregating with other species.

Aggregating with individuals of other species is a remarkable behavior because species differ in many phenotypic traits. These include physiological requirements, the way they deal with natural enemies, the type of food they consume, the level of aggressiveness they show towards conspecifics and heterospecifics, and how they communicate. Therefore, aggregating with other species requires that individuals overcome at least some of these differences, so that they can recognize, tolerate, and perhaps cooperate with each other (Cocroft, 2001; Boulay et al., 2019). For example, two species of web-building spiders, *Hypochilus thorelli* (Hypochilidae) and *Achaearanea tepidariorum* (Theridiidae), form mixed-species aggregations (MSA) in rock outcrops (Hodge and Storfer-Isser, 1997). Before grouping, individuals of one species need to use chemical and vibrational cues to recognize that silk threads were laid by individuals of another species. This task implies that these two non-closely related species share some communication channels for receiving cues and/or sending signals. Moreover, individuals must have the neural and cognitive mechanisms to interpret that the other species is not a potential predator. Finally, if individuals of one species build their own webs using the web of other species to anchor silk threads, some level of behavioral flexibility and tolerance are necessary.

Variation in phenotypic traits across species may either favor or prevent the formation of MSA (Cocroft, 2001; Gerhold et al., 2015; Perón, 2017; Boulay et al., 2019). Phenotypic variation is related, at least in part, to the phylogenetic relationship between the species that compose MSA. For instance, closely related species are more likely to have the same communication channels, which may favor both the recognition and exchange of information. This is the case of mixed-species bird flocks, which are thought to be maintained because species have similar communication channels. The vocal signals produced by one or more species in the presence of a potential predator (e.g., a hawk) are easily recognized and interpreted as an alarm signal by all species in the group (Goodale et al., 2020). However, closely related species may also have similar sizes and diets, so their trophic niches may overlap. In this situation, the close relationship between species may lead to intense interspecific competition, which ultimately may prevent the formation of MSA. In fact, members of mixed-species bird flocks that follow army-ants show clear differences in body size, gape size, and types of prey, suggesting that competition for food is an important factor to the composition of these flocks (Powell, 1985; Sridhar et al., 2009).

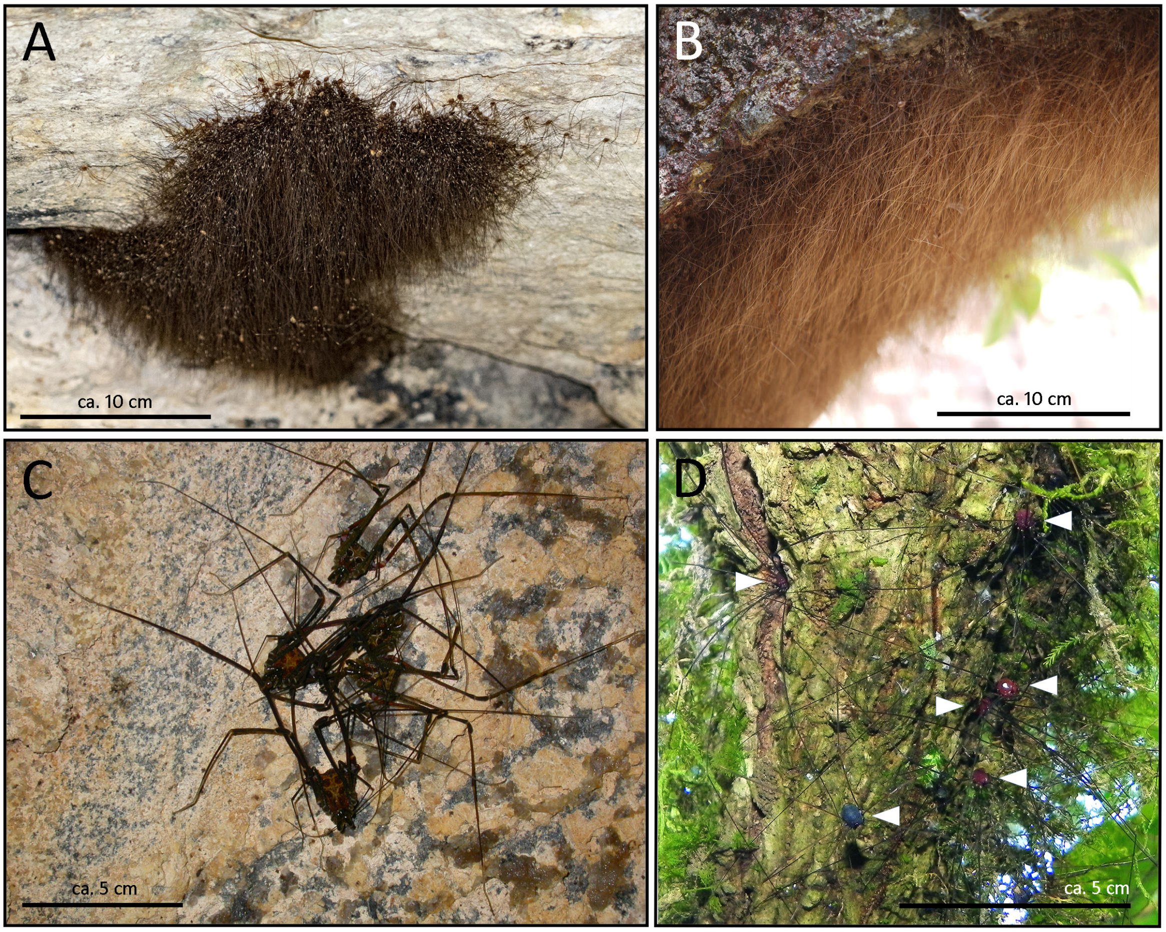


FIGURE 1 | Types of aggregations recorded in harvestmen. **(A)** Dense single-species aggregation of an unidentified sclerosomatid (Eupnoi) in Texas, United States of America (photo: Aleksomber, Wikimedia Commons). **(B)** Dense single-species aggregation of an unidentified sclesomatid in Thane, India (photo: Dinesh Valke, Wikimedia Commons). **(C)** Loose single-species aggregation of *Acutisoma longipes* (Laniatores) inside a cave in southeastern Brazil (photo: G. Machado). **(D)** Loose mixed-species aggregations of three species of *Prionostemma* (Eupnoi) from Costa Rica (photo: I. Escalante). White arrows indicate individuals of different species that can be recognized based on body color.

of Science and Google Scholar, our backward and forward literature search resulted in 12 cases of MSA in harvestmen (**Table 1**, including the original data presented in this study). This frequency is higher than in any insect order reported in Boulay et al. (2019). The number of species found in MSA ranges from 2 to 7, and the number of individuals ranges from 2 to 356 (**Table 1**). In some cases, one or two species are consistently more frequent than the other species in the MSA (e.g., Machado and Vasconcelos, 1998; Elpino-Campos et al., 2001; Pereira et al., 2004), but it is not a general rule. Five cases of MSA involve only species of Eupnoi, six involve only species of Laniatores, and one case involves species of the two suborders (**Table 1**). These MSA are always loose, and the roosting sites are usually humid places protected from direct sunlight (**Table 1**).

All studies on MSA in harvestmen are descriptive and none of them investigate benefits or costs of gregariousness. To stimulate further research on this subject, we provide a comprehensive review of the possible benefits and costs of MSA. Some hypotheses on the benefits of MSA in harvestmen have already been proposed (e.g., Machado and Vasconcelos, 1998; Machado and Macías-Ordóñez, 2007), but here we include new ideas. Regarding the costs associated with MSA, we provide for the first time a set of hypotheses that may guide future empirical studies on the subject. Finally, we stress that there is no available information on whether harvestmen cooperate while forming and/or maintaining the aggregations. We know, however, that at least in one species of harvestman individuals mark the roosting sites, potentially by using recruitment pheromones (Donaldson and Grether, 2007; Grether et al., 2014b), and this type of chemical communication may allow cooperative behaviors to evolve (reviewed in Prokopy and Roitberg, 2001). Thus, although our review does not assume cooperation among individuals, we explore how chemical signals may favor the formation of MSA in the section "Gregariousness in *Prionostemma* harvestmen" below. Additionally, we invite future research to explore cooperation in harvestman SSA or MSA, which is beyond the scope of our project.

Benefits of Mixed-Species Aggregations

In general terms, the benefits of joining MSA can be divided into four main categories, which are similar to that already reported for SSA (Ward and Webster, 2016): physiological benefits, defense benefits, foraging benefits, and reproductive benefits (**Table 2** and **Figure 2**). Although there are other types of benefits, we selected only those that are most frequently cited in the recent literature on MSA in arthropods and vertebrates (Boulay et al., 2019; Goodale et al., 2019, 2020). Moreover, we focused only on

TABLE 1 | Cases of mixed-species aggregations in harvestmen (Arachnida: Opiliones).

Taxa	% of individuals of each species (aggregation size)	Roosting site (country)	Source
Acanthopachylus aculeatus + *Pachyloides thorellii* (Gonyleptidae)	Many + Few (NA)	Under rocks and rotting logs (Uruguay)	Capocasale and Bruno-Trezza, 1964
Discocyrtanus oliverioi + *Discocyrtus* sp. + *Mischonyx cuspidatus* (Gonyleptidae)	17 + 9.5 + 73.5 (8–66)	Under rocks and rotting logs (Brazil)	Elpino-Campos et al., 2001; Pereira et al., 2004
Discocyrtus sp. 1 + sp. 2 + *Geraecormobius* sp. + *Mischonyx cuspidatus* + Tricommatinae (Gonyleptidae)	NA (NA)	Under rocks and rotting logs (Brazil)	Mestre and Pinto-da-Rocha, 2004
Discocyrtus testudineus + *Hernandaria scabricula* (Gonyleptidae) + *Gryne orensis* + *Metalibitia argentina* (Cosmetidae) + *Holmbergiana weyenberghi* (Sclerosomatidae)	NA (NA)	Cavities in the ground and under rotting logs (Argentina)	Martínez, 1974
Encheiridium montanum + *Eugyndes* sp. + *Holoversia nigra* (Gonyleptidae)	50 + 4.8 + 45.2 (5–34)	In the base of clumps of roots in a swamp (Brazil)	Machado and Vasconcelos, 1998
Leiobunum flavum + *L. vittatum* (Sclerosomatidae)	NA (NA)	Under the leaves of a camp-ground shelter (United States)	Cockerill, 1988
Leiobunum flavum + [*L. vittatum* + *L. townsendi*] (Sclerosomatidae)	90 + [10] (25–300)	Under the leaves of a camp-ground shelter (United States)	Cockerill, 1988
Phareicranaus calcariferus (Cranaidae) + *Santinezia* sp. (Cranaidae)	NA (8–33)	Fallen palm frond sheaths (Trinidad)	Townsend et al., 2009
Platybunus bucephalus + *Rilaena triangularis* (Phalangiidae)	NA (NA)	Trunk crevices, under rotting logs and stones (France)	Parisot, 1962
Prionostemma sp. 1 + sp. 2 (Sclerosomatidae)	29 + 71 (2–315)	Spiny palms (Nicaragua)	Harvey et al., 2017
Prionostemma sp. 5 + sp. 6 + sp. 7 + sp. 8 + sp. 9 + sp. 10 + sp. 11 (Sclerosomatidae)	Highly variable (2–16)	Tree trunks, mossy branches, arborescent ferns (Costa Rica)	This study
Serracutisoma proximum + *S. spelaeum* (Gonyleptidae)	66–31 (4–81)	Inside caves (Brazil)	Chelini et al., 2012

All cases are restricted to representatives of two suborders: Eupnoi (families Phalangiidae and Sclerosomatidae) and Laniatores (families Cranaidae, Cosmetidae, and Gonyleptidae). NA, non-available information.

TABLE 2 | Comparison of the benefits associated with single-species aggregations (SSA) and mixed-species aggregations (MSA).

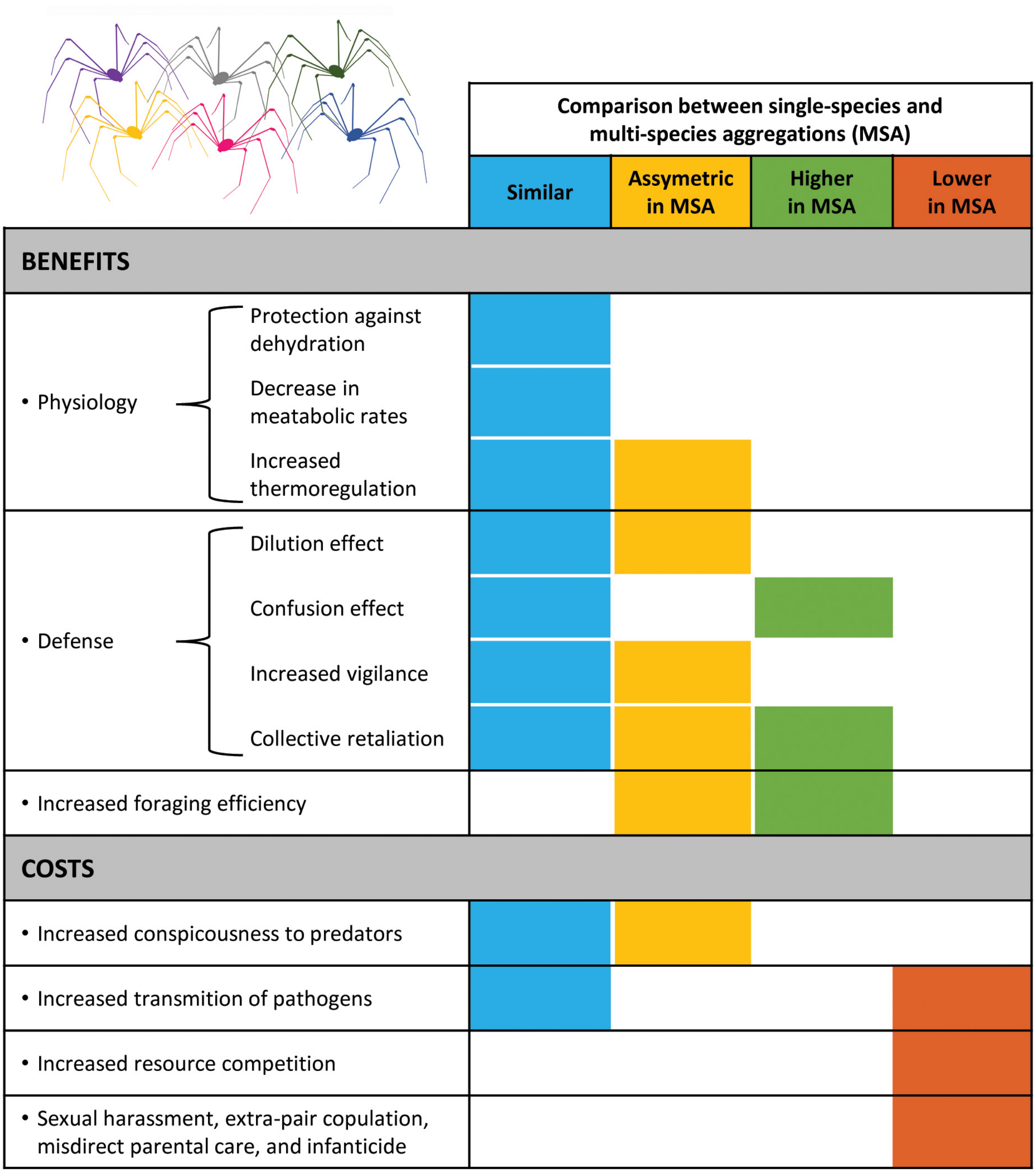
Types of benefits	Comparison between SSA and MSA
Physiology	
(1) Protection against dehydration	(1a) Benefits should be **similar** if the density of the aggregated individuals is high in both types of aggregations
(2) Reduction of metabolic rates	(2a) Benefits should be **similar** if the effect is based solely on the presence of other individuals nearby
(3) Thermoregulation	(3a) Benefits should be **similar** if individuals of all species have similar rates of heat production or capacity of heat conservation (3b) Benefits should be **asymmetric in MSA** if species differ in heat production rate or heat conservation capacities
Defense	
(4) Dilution effect	(4a) Benefits should be **similar** if individuals of all species are equally vulnerable to predation (i.e., when they have similar body sizes, coloration, defense mechanisms, escape speed, etc.) (4b) Benefits should be **asymmetric in MSA** if individuals of some species are more likely to be singled out by predators than individuals of other species
(5) Confusion effect	(5a) Benefits should be **similar** if individuals of all species are morphologically and behaviorally similar (5b) Benefits should be **higher in MSA** if phenotypic variation among aggregated species may disrupt the search image of predators even further
(6) Increased vigilance	(6a) Benefits should be **similar** if individuals of all species are equally efficient and engaged in detecting and announcing the presence of a predator (e.g., releasing alarm pheromones) (6b) Benefits should be **asymmetric in MSA** if individuals of some species may parasitize/eavesdrop the signals (e.g., alarm pheromones) produced predominantly or exclusively by individuals of other species
(7) Collective retaliation	(7a) Benefits should be **similar** if individuals of all species are equally efficient and engaged in repelling predators (7b) Benefits should be **higher in MSA** if the presence of individuals of different species with different defense mechanisms somehow improve the probability of repelling predators (this benefit probably does not apply to harvestmen) (7c) Benefits should be **asymmetric in MSA** if individuals of some species may parasitize the defensive effort of individuals of other species
Foraging	
(8) Improved resource location or acquisition	(8a) Benefits should be **higher in MSA** if the presence of individuals of different species somehow improves resource acquisition (this benefit does not apply to harvestmen) (8b) Benefits should be **asymmetric in MSA** if individuals of some species are better at exploring food resources or if individuals of some species parasitize the foraging effort of individuals of other species (this benefit does not apply to harvestmen)
Reproduction	
(9) Increased mating success	(9a) Benefits (if any) should **apply only to SSA** because the mating success of individuals of one species does not increase in the presence of individuals of other species, unless the sexual pheromones are similar between species (this benefit probably does not apply to harvestmen)

Comparisons can have three outcomes: (1) Similar, when the benefits of joining SSA and MSA are similar for all species; (2) Asymmetric, when the benefits of joining SSA are qualitatively or quantitatively different from joining MSA, but only for a subset of the species that form the MSA; and (3) Higher, when the benefits of joining MSA are higher than joining SSA. For the sake of simplicity, our comparisons use a mean-field approach, according to which the benefits are described in terms of means for each species, ignoring within-species variations. The outcome of the comparisons is highlighted in bold.

the benefits that can be supported by our knowledge on harvestman behavior, ecology, and physiology. For instance, we do not consider collective hunting as a possible benefit because aggregated harvestmen are always stationary and quiescent (Machado and Macías-Ordóñez, 2007). Additionally, after individuals disperse from the roosting site at night, they forage alone (Machado et al., 2000), and no coordinated collective hunting has ever been reported in harvestmen (Acosta and Machado, 2007).

Before expanding on the benefits, we stress that MSA may simply be a consequence of individuals of different species being independently attracted to places with specific features (Rasa, 1990). This explanation, known as the "similar habitat hypothesis" (Quinn et al., 2003), requires high tolerance to conspecifics and heterospecifics (Boulay et al., 2019). In this sense, harvestmen are unusual arachnids because cannibalism among subadults and adults has never been reported under field conditions. Most records of cannibalism include adults eating eggs or early instars (Acosta and Machado, 2007). Moreover, despite anecdotal records of heterospecific predation in harvestmen (Cokendolpher and Mitov, 2007), this behavior is rare and probably occurs when there is a great size difference between individuals of each species. Thus, contrary to spiders, whip-spiders, and scorpions, conspecifics and heterospecifics pose low risks of predation, which may have favored tolerance and the evolution of gregariousness in harvestmen (Machado, 2002).

Is there any evidence that the similar habitat hypothesis applies to MSA in harvestmen? As we mentioned earlier, most harvestmen have low tolerance to dehydration, and thus aggregations are usually found in humid places with low light incidence. Even in the cavernicolous habitat, which is humid and dark, SSA of *Acutisoma longipes* (Gonyleptidae) usually occur close to the river that crosses the cave and away from cave



		Comparison between single-species and multi-species aggregations (MSA)			
		Similar	Assymetric in MSA	Higher in MSA	Lower in MSA
BENEFITS					
• Physiology	Protection against dehydration	🟦			
	Decrease in meatabolic rates	🟦			
	Increased thermoregulation	🟦	🟨		
• Defense	Dilution effect	🟦	🟨		
	Confusion effect	🟦		🟩	
	Increased vigilance	🟦	🟨		
	Collective retaliation	🟦	🟨	🟩	
• Increased foraging efficiency			🟨	🟩	
COSTS					
• Increased conspicousness to predators		🟦	🟨		
• Increased transmition of pathogens		🟦			🟧
• Increased resource competition					🟧
• Sexual harassment, extra-pair copulation, misdirect parental care, and infanticide					🟧

FIGURE 2 | Comparison of benefits and costs of single-species aggregations (SSA) and mixed-species aggregations (MSA). This figure summarizes the reasoning presented in **Tables 2**, **3**, where there are four possible outcomes: (1) Similar, when the benefits–costs of joining SSA and MSA are similar; (2) Asymmetric, when the benefits–costs of joining SSA are qualitatively or quantitatively different from joining MSA, but only for a subset of the species that form the MSA; (3) Higher, when the benefits–costs of joining MSA are higher than joining SSA; and (4) Lower, when the benefits–costs of joining MSA are lower than joining SSA.

openings (Machado et al., 2000), suggesting that aggregations are not randomly distributed in the habitat. This pattern was not observed in two gonyleptid species (*Serracutisoma proximum* and *S. spelaeum*) closely related to *A. longipes* that also live inside caves and form MSA. Although these two species are congeneric, aggregations of *S. proximum* occur more frequently on walls close to cave openings (where individuals are exposed to light incidence, higher temperature, and humidity fluctuation) than aggregations of *S. spelaeum* (Chelini et al., 2012). This finding does not support the notion that MSA in harvestmen are a consequence of similar and specific habitat requirements by different species. However, MSA frequently involve species belonging to the same genus (**Table 1**), which are likely to have similar physiological and ecological requirements (Chown and Nicolson, 2004). This high frequency of congeneric species in MSA cannot be explained by the composition of the local harvestman communities, which usually include a great diversity of genera (Curtis and Machado, 2007). Thus, until a formal test of the similar habitat hypothesis is available, it may be premature to discard it as a possible (but certainly not the only) explanation for the formation of MSA in harvestmen.

Mixed-species aggregations can also be a consequence of limited resource availability, so different species are forced to share the same places (Rasa, 1990). For instance, individuals of several fish species are associated with sea anemones, which provide protection to small-bodied species or small individuals within species. Considering that colonies of sea anemones are highly clumped, the spatial distribution of different fish species follows the availability of the anemones, resulting in the formation of MSA (Brooker et al., 2019). This explanation, known as the "resource limitation hypothesis," assumes that individuals of different species share similar requirements (e.g., protection) and that the risk of antagonistic interactions (i.e., cannibalism and predation) is low. The best candidate for limiting resources favoring the formation of MSA in harvestmen is the roosting site. Although biologically plausible, there is no evidence supporting that roosting sites are a limiting resource for harvestmen. Studies on two species of *Prionostemma* (Sclerosomatidae) that form SSA in Central America show no preference for different palm trees, which are the main roosting sites (Donaldson and Grether, 2007; Grether and Donaldson, 2007; Teng et al., 2012; Grether et al., 2014a,b). This finding contradicts the existence of suitable roosting sites with low availability in the field. Thus, we argue that the resource limitation hypothesis is unlikely to be an explanation for the existence of harvestman aggregations (SSA or MSA).

Physiological Benefits

Given the weak support for two simple explanations (i.e., similar habitat and resource limitation hypotheses) that require no individual benefit for the existence of MSA, we argue that this unusual form of group living evolved and is maintained in harvestmen because it provides benefits. Here we discuss three *physiological* benefits: protection against stressful abiotic conditions, reduction of metabolic rates, and thermoregulation (**Table 2**).

Gregariousness may act as a behavioral mechanism to reduce water loss in many arthropods (isopods: e.g., Friedlander, 1965; millipedes: e.g., Dangerfield, 1993; insects: e.g., Danks, 2002). In harvestmen, the close body contact and the intertwining of legs may reduce airflow and thus individual water loss (Machado and Macías-Ordóñez, 2007). This may be the case for the dense SSA observed in some Eupnoi from xeric regions composed of thousands of quiescent individuals (e.g., Wagner, 1954; **Figures 1A,B**). However, all cases of MSA in harvestmen are loose (**Table 1**), which is unlikely to protect the individuals from dehydration because they are not so densely packed together to prevent water loss. Thus, although loose aggregations may also promote changes in microclimatic conditions, we anticipate that the potential benefits they provide in terms of protection against dehydration should be lower than in dense aggregations.

Aggregations have also been found to reduce the resting metabolic rates of some insects (reviewed in Chown and Nicolson, 2004). Such reduction may be beneficial because it decreases energy expenditure and spiracular water loss in tracheate arthropods, including arachnids (Hadley, 1994). There is one single study showing that *Vonones ornatus* (Cosmetidae) harvestmen in small and loose aggregations have reduced metabolic rates (Anderson, 1993). The exact mechanism linking gregariousness and metabolic rates in harvestmen has not been explored yet and certainly deserves attention. Another important question is whether the reduction in metabolic rates reported for loose SSA of *V. ornata* also occurs in loose MSA of other harvestman species. Therefore, this potential benefit cannot be discarded and deserves further consideration.

Finally, gregariousness may favor thermoregulation by improving heat production and/or conservation, protecting the individuals from cold conditions. This benefit is particularly important among endothermic vertebrates living in high latitudes, where the temperature may reach extremely low values (Gilbert et al., 2010). Among insects that live in less harsh habitats, gregariousness may also play an important role in their thermal ecology (Danks, 2002; Chown and Nicolson, 2004). For instance, caterpillars maintain higher and more stable body temperatures when aggregated (e.g., Casey et al., 1988; Joos et al., 1988; Casey, 1993; Fitzgerald, 1993), which leads to higher growth rates (e.g., Scriber and Lederhouse, 1983; Knapp and Casey, 1986). Similarly, temperatures are higher and buffered in aggregations of the lady beetle *Coleomegilla maculata* (Benton and Crump, 1979). Although there is no information on the temperature inside harvestman aggregations, we argue that dense aggregations may conserve heat and buffer temperature variations, at least in its core. Heat control and temperature buffering would be particularly beneficial for individuals in overwintering aggregations from cold regions (e.g., Holmberg et al., 1984; Novak et al., 2004) and in diurnal aggregations from hot xeric regions (e.g., Wagner, 1954). Nonetheless, since all cases of MSA in harvestmen are loose (**Table 1**), we consider that a thermoregulation benefit is unlikely.

Defense Benefits

Aggregations in harvestmen may provide several defense benefits against predators. At least among Laniatores, scent gland secretions released by the individuals are a powerful chemical deterrent that repel different types of predators, including invertebrates and small vertebrates (e.g., Eisner et al., 2004; Machado et al., 2005). Thus, a first defense benefit of gregariousness is a higher amount of secretion released upon disturbance — a collective retaliation that may improve the efficiency of the chemical defenses (Machado and Macías-Ordóñez, 2007; **Table 2**). In the case of MSA, behavioral observations show that some species rarely release scent gland secretions (Elpino-Campos et al., 2001; Pereira et al., 2004). In one report, the most common species in the aggregation does not even produce scent gland secretions (Machado and Vasconcelos, 1998). This finding can be explained by the "protector species hypothesis," according to which individuals of a poorly defended species benefit by associating with individuals of a well-defended species that aggressively repels potential predators of both species (Sullivan, 1984; Quinn et al., 2003; Goodale et al., 2014). The protector species hypothesis assumes that the benefits of MSA in terms of collective retaliation are necessarily asymmetric because not all species contribute to repel the predators (**Table 2**).

In known cases of MSA in harvestmen, the poorly defended species (e.g., *Mischonyx cuspidatus* and *Encheiridium montanum*) comprise most part of the individuals in the aggregation, whereas the well-defended species (e.g., *Discocyrtanus oliverioi* and *Holoversia nigra*) account for only a small number of individuals (Machado and Vasconcelos, 1998; Elpino-Campos et al., 2001; Pereira et al., 2004; **Table 1**). It is possible that the well-defended species are not even gregarious. In this case, well-defended species may serve as attractors (i.e., initiators) to poorly defended species (i.e., followers) that would aggregate around the former. This hypothesis does not imply that initiators are deliberately attracting followers using recruitment pheromones, although it may be the case. If initiators have some benefit when followers aggregate around them, it would be advantageous to release recruitment pheromones. An obvious advantage for the initiators of attracting followers is a decreased probability of being singled out by predators (Sridhar et al., 2009). This "dilution effect" (Turner and Pitcher, 1986; **Table 2**) is another defense benefit attributed to harvestman aggregations (Machado and Macías-Ordóñez, 2007). In MSA, both initiators and followers may enjoy this defense benefit; if the benefit is similar to all species in the aggregation, is an open question that deserves future studies.

Harvestmen also obtain the benefit of having an early alarm signal in aggregations. The scent gland secretions released by aggregated individuals and their movement to escape from an attack work as an alarm — one chemically and the other mechanically mediated (Machado et al., 2002). Experimental field evidence shows that aggregations of *Serracutisoma gnaspinii* (Gonyleptidae) with a greater number of individuals disperse faster to the chemical alarm promoted by the emission of scent gland secretions (Machado et al., 2002). Large harvestman aggregations have more sensory legs full of chemoreceptors to perceive the scent gland secretions released by other individuals. Due to the alarm communication, aggregations may increase both the escape capability of the individuals and their probability to survive a predatory attack (Machado and Macías-Ordóñez, 2007). In MSA, there is observational evidence showing that scent gland secretions released by one species may work as a chemical alarm to individuals of other species that do not release scent gland secretions (Machado and Vasconcelos, 1998). This suggests that the benefits promoted by the alarm communication are asymmetric: while individuals of species that do not release scent gland secretions benefit from the chemical alarm released by other species, the opposite does not happen (**Table 2**). Regarding the mechanically mediated alarm, however, all species in MSA may be favored because it only requires that individuals bump each other while they are dispersing from the aggregation (i.e., "Trafalgar effect"; Treherne and Foster, 1981).

Finally, it is possible that the fast dispersion of aggregated individuals causes a confusion effect in the search image of visually oriented predators (Goodale et al., 2019; **Table 2**). Most examples of confusion effect have been reported for vertebrate prey, but experiments with aggregations of the water flea *Daphnia magna* showed that stickleback attack rates decline when aggregated individuals are in larger aggregations, are closer together, have similar body size, and are moving parallel to each other (Ohguchi, 1981). After disturbance, individuals in harvestman aggregations quickly disperse, running away or falling from the roosting site (Newman, 1917; Holmberg et al., 1984). Certain species of Eupnoi also exhibit bobbing behavior (Holmberg et al., 1984), which is a fast up and down body movement (Gnaspini and Hara, 2007). The collective movement of dozens to thousands of individuals bobbing probably confuses the identification and precise location of a potential prey's body by the predator (Escalante et al., 2019). This confusion effect may be more accentuated in the MSA of sclerosomatids in Central America because individuals of different species show great variation in body color (**Figures 1D**, **3**), which may disrupt even more the search image of potential predators (**Table 2**).

Reproductive Benefits

Aggregations during or immediately before the beginning of the breeding season may ensure that individuals of both sexes will be in close proximity. Thus, aggregations may provide mating benefits by increasing the opportunities to gain access to mates and by reducing the mating search costs (Ward and Webster, 2016). However, there is no evidence that harvestman aggregations are related to reproduction (Machado and Macías-Ordóñez, 2007). In many cases, aggregations occur during the winter, when no breeding activity occurs (e.g., Holmberg et al., 1984; Machado et al., 2000; Novak et al., 2004; Willemart and Gnaspini, 2004; Chelini et al., 2012). When aggregations occur during the breeding season, sexual interactions have not been observed close to them (e.g., Edgar, 1971; Cockerill, 1988; Grether et al., 2014a). In the case of MSA, the possibility of mating benefits is non-existent because individuals derive no reproductive advantage from aggregating with heterospecifics.

Costs of Mixed-Species Aggregations

In general terms, the costs of joining MSA can be divided into four main categories, which are similar to those already reported for SSA (Ward and Webster, 2016): increased conspicuousness, increased transmission of pathogens and parasites, increased resource competition, and costs related to reproduction, such as increased risk of sexual harassment and extra-pair copulation, misdirect parental care, and infanticide (**Table 3** and **Figure 2**). This list includes only the costs that are most frequently explored in the recent literature on MSA in arthropods and vertebrates (Boulay et al., 2019; Goodale et al., 2019, 2020). Because harvestman aggregations have no connection with reproduction (Machado and Macías-Ordóñez, 2007), including parental care, we do not consider that MSA can impose reproductive costs. However, for species in which aggregations are somehow connected to reproduction, as occurs with many bird species (examples in Goodale et al., 2017; Boulay et al., 2019), we expect the costs of group living would be lower in MSA than in SSA (**Figure 2**).

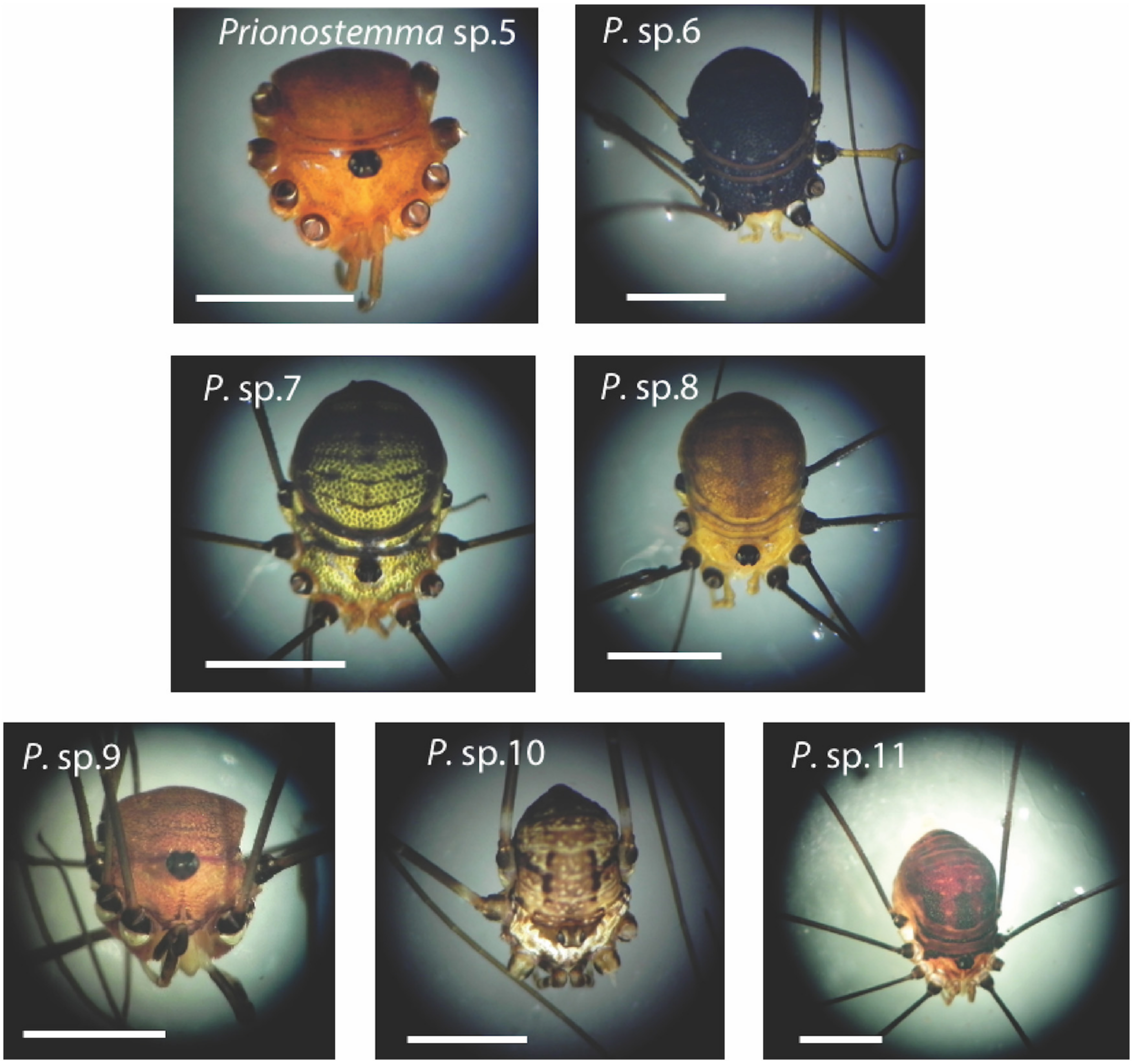


FIGURE 3 | The seven species of *Prionostemma* harvestmen studied in a tropical forest of Costa Rica. These species are currently undescribed, but can be easily recognized based on external features, such as body size and color, as well as relative leg length and color. To reinforce our criteria of species recognition, we also found differences between species in the male genital morphology. Scale bars = 3 mm.

Increased Conspicuousness

Gregariousness may increase the chances of detection by predators because aggregations may be visually or chemically more conspicuous than isolated individuals (Vulinec, 1990; **Table 3**), especially if individuals are colorful, as it is the case of some *Prionostemma* species (**Table 1** and **Figures 1D**, **3**). To date, we lack information about whether harvestman aggregations (SSA or MSA) increase conspicuousness and consequently predatory attacks. However, if aggregations increase conspicuousness and the risk of predatory attacks, we predict that individuals of cryptic species should avoid joining MSA with conspicuous species. In this situation, individuals of cryptic species would derive more benefits by aggregating exclusively with conspecifics because they would make the aggregation less conspicuous to visually oriented predators. On the contrary, individuals of conspicuous species should infiltrate aggregations of cryptic species where they would be more protected from visually oriented predators than in aggregations composed only of conspecifics. By doing so, individuals of conspicuous species would 'break' the aposematic value of the aggregations. This conflict between individuals of different species is an interesting, yet unexplored, idea that may have important implications for the patterns of species co-occurrence in MSA.

Transmission of Pathogens and Parasites

One of the most obvious costs of gregariousness is the increased transmission of internal and external pathogens and parasites (Côté and Poulin, 1995; Kappeler et al., 2015). Harvestmen are exposed to a great sort of pathogens and parasites (reviewed in Cokendolpher and Mitov, 2007). The group of endoparasites most frequently reported for harvestmen are gregarines, which

TABLE 3 | Comparison of the costs associated with single-species aggregations (SSA) and mixed-species aggregations (MSA).

Types of costs	Comparison between SSA and MSA
Predation	
(1) Increased conspicuousness	Costs should depend on the conspicuousness of the most common species in the aggregation: (1a) Costs should be **similar** if all species are equally conspicuous (1b) Costs should be **asymmetric** if species differ in conspicuousness: inconspicuous species will experience higher costs when aggregated with conspicuous species
Parasitism	
(2) Increased pathogens and parasites transmission	Costs should depend on how species-specific the pathogens and parasites are: (2a) Costs should be **lower in MSA** if pathogens and parasites are highly species-specific, because the number of conspecifics nearby is reduced (2b) Costs should be **similar** if parasites are not species-specific
Competition	
(3) Increased resource competition	(3a) Costs should be **lower in MSA** because interspecific variation in diet and foraging behavior may promote niche differentiation and decrease resource competition (this cost probably does not apply to harvestmen)
Reproduction	
(4) Sexual harassment, extra-pair copulation, misdirect parental care, and infanticide	• Costs should be **lower in MSA** because the probability of sexual harassment, extra-pair paternity, misdirect parental care and infanticide is lower when individuals of one species are surrounded by individuals of other species (this cost does not apply to harvestmen)

Comparisons can have four outcomes: (1) Similar, when the costs of joining SSA and MSA are similar for all species; (2) Asymmetric, when the costs of joining SSA are qualitatively or quantitatively different from joining MSA, but only for a subset of the species that form the MSA; (3) Higher, when the costs of joining MSA are higher than joining SSA; and (4) Lower, when the costs of joining MSA are lower than joining SSA. For the sake of simplicity, our comparisons use a mean-field approach, according to which the costs are described in terms of means for each species, ignoring within-species variations. The outcome of the comparisons is highlighted in bold.

are apicomplexans that infect the digestive tract of their hosts. A harvestman infects itself when the small oocysts, present in the feces of other individuals, attach to the tip of its legs and then are ingested during leg grooming (Cokendolpher and Mitov, 2007). Considering that aggregated individuals share the same roosting sites and probably defecate in or close to these sites, it is likely that harvestman aggregations increase the chances of infection by gregarines. Unfortunately, there is no information on host specificity for the gregarines that infect harvestmen. If the host-parasite relationship is not species-specific (e.g., Cokendolpher, 1993), the risk of contamination may be equally high in SSA and MSA. On the contrary, if there is some degree of specialization in the host-parasite relationship, the risk of infection should be lower in MSA than in SSA (**Table 3**).

Harvestmen in aggregations are also exposed to external parasites, including many species of *Leptus* mites (Cokendolpher and Mitov, 2007). These mites deposit a cone of cementing material at the attachment site that, along with their mouthparts, form a tight anchorage on the tegument of the host (Åbro, 1988). Thus, while attached, it is very unlikely that one harvestman carrying mites will infect others. However, we observed mites moving around on the body of *Prionostemma* harvestmen that form MSA (see section "Costs of gregariousness" below). This indicates that mites do not attach their mouthparts immediately to their hosts, and thus may move from one host to another. Currently, there is no information on how long it takes for a *Leptus* mite to climb the host and attach its mouthparts. We know, however, that many *Leptus* mites prefer certain body parts on their harvestman hosts (e.g., McAloon and Durden, 2000; Townsend et al., 2008), which implies that they move around some time until they find a proper attachment site. The main implication of this movement is that horizontal transmission of mites among aggregated harvestmen is possible. It seems that *Leptus* mites do not show great host specialization because the same species may parasitize numerous harvestman species (Cokendolpher and Mitov, 2007). Therefore, the costs of infection are likely to be similar in SSA and MSA.

Resource Competition

When dozens, hundreds or even thousands of individuals aggregate in the same site, they may compete for resources, mainly food. However, when harvestman aggregations disperse at night, individuals visit different places and can move long distances (e.g., Grether and Donaldson, 2007). When individuals of *Acutisoma longipes* and *Serracutisoma spelaeum* leave the cave at night, each one follows a specific trail to forage on substrates as different as the external cave walls and the canopy of the forest surrounding the cave (Gnaspini, 1996; Machado et al., 2000). Since the foraging area shows little overlap between individuals, it is unlikely that they compete for food. We do not know if this also happens in large aggregations of Eupnoi that may have between 70,000 (Wagner, 1954) and 300,000 individuals (Mukherjee et al., 2010) in habitats with low productivity, such as deserts and cold forests. It is important to note, however, that harvestmen are highly generalist feeders and forage on many different items, including live and dead animals, as well as vegetal and fungi matter (Acosta and Machado, 2007). Thus, competition for food is not expected to have an important role in harvestmen ecology (Curtis and Machado, 2007). Supporting this claim, a long-term study on harvestman communities in deciduous woods from England found no evidence for resource competition among species (Adams, 1984). If competition is important, joining MSA may attenuate it, considering that differences in size, morphology, and microhabitat use between species somehow reflect differences in their food niche. This hypothesis has been originally proposed for MSA of birds that forage together (Powell, 1985; Sridhar et al., 2009), and to our knowledge, there is no demonstration that it also applies to invertebrates. Harvestmen offer an opportunity to test this hypothesis because of the numerous cases of SSA and MSA, which allow exploring how the intensity of competition for food varies according to the type of aggregation while controlling for aggregation size.

STUDY CASE: MIXED-SPECIES AGGREGATIONS IN *Prionostemma* HARVESTMEN

Here, we provide empirical data on gregariousness in harvestmen of the genus *Prionostemma*, whose species form both SSA (e.g., Coddington et al., 1990; Grether and Donaldson, 2007; Teng et al., 2012) and MSA (e.g., Grether et al., 2014a). Our study system includes seven currently undescribed species that occur in primary and secondary forests in southwestern Costa Rica. Since a previous study refers to other four undescribed species from northeastern Costa Rica as *Prionostemma* sp.1 to sp.4 (Proud et al., 2012), we will refer to the species studied here as *Prionostemma* sp.5 to sp.11 (**Figure 3**). We are confident that the seven species studied are different taxonomic entities because they show clear differences in several traits (body size and color, relative leg length, and specially the morphology of male genitalia) commonly employed in the taxonomy of Neotropical sclerosomatids belonging to the subfamily Gagrellinae (e.g., Tourinho-Davis and Kury, 2003; Tourinho et al., 2015).

Natural History Background

The *Prionostemma* from Costa Rica are found during the day either solitarily or forming aggregations, mostly beneath moss, on tree buttresses, inside tree crevices, and on palm and ginger leaves. At dusk, individuals disperse and are found foraging and searching for mates on plants and on the forest floor (Domínguez et al., 2016; Escalante and Elias, 2021). Individuals of other *Prionostemma* species from Nicaragua can move long distances when they leave their diurnal roosts. For instance, Grether and Donaldson (2007) recaptured individuals 130 m from the roosting sites where they were originally marked. Although the roosting sites in Nicaragua were predictable, the membership of aggregations was fluid because individuals were recaptured in different roosting sites over time (Donaldson and Grether, 2007). Similar results were obtained for one of the Costa Rican species studied here, *Prionostemma* sp.5, which also showed low levels of roosting site fidelity (Escalante and Elias, 2021).

As occurs with many species of Eupnoi (e.g., Guffey, 1999; Houghton et al., 2011; Powell et al., 2021a,b), individuals of *Prionostemma* are frequently found missing legs in the field (Escalante et al., 2013, 2020, 2021; Domínguez et al., 2016). This is an indication of autotomy (i.e., the voluntary release of legs to escape predatory attempts) and to a much lesser extent the effect of failed molt (Gnaspini and Hara, 2007; Emberts et al., 2019). Autotomy affects the locomotor performance and the energetics of locomotion of sclerosomatid harvestmen (Escalante et al., 2013, 2020, 2021; Domínguez et al., 2016). This reduced locomotor performance may affect the chances of surviving future encounters with predators. Thus, the perception of predation risk is likely to change after autotomy (Emberts et al., 2019), and individuals may change their anti-predator behaviors (Fleming et al., 2007). For instance, in a laboratory experiment with the wolf spider *Schizocosa avida*, autotomized individuals showed increased avoidance of olfactory cues of predators (scorpions) when compared with intact individuals (Punzo, 1997). If aggregations of *Prionostemma* harvestmen indeed increase the protection of the individuals against predators, a possible behavioral response of autotomized individuals would be a tendency to roost more frequently in aggregations instead of solitarily.

Another conspicuous feature of the biology of many harvestman species is the high prevalence of erythraeid mites (Mesostigmata), which are ectoparasites of many arthropods (Cokendolpher and Mitov, 2007). In *Prionostemma* from Nicaragua, the prevalence of *Leptus* mites changed among roosting sites and species (Grether et al., 2014a), but no comparison between solitary and aggregated individuals was conducted. Although we know the prevalence of ectoparasite mites in several harvestman species (e.g., McAloon and Durden, 2000; Mitov, 2000; Townsend et al., 2006, 2008; Grether et al., 2014a), there is no information on the negative fitness-related effects these mites may have on their hosts. In other arthropod taxa, however, the infection by erythraeid mites has clear negative effects on their hosts. In *Drosophila*, for instance, mites extract hemolymph from the host, causing marked cuticular damage during feeding. The long-term nutrient extraction and mite-derived damages have negative effects on the reproductive tissues of males and females (Polak, 1996; Benoit et al., 2020). In harvestmen, *Leptus* mites extract hemolymph from their hosts, and the attachment of mites promotes intense immune response in their hosts (Åbro, 1988). Evidence from other arthropods indicates that activation of the immune system is costly, imposing several fitness trade-offs that may reduce reproductive performance and/or survival (Schmid-Hempel, 2005). Thus, it is reasonable to suppose that mite infestation is costly to harvestmen as well.

Objectives

Our first goal in this empirical part of the study is to describe gregariousness in seven *Prionostemma* species. More specifically, we report: (a) the frequency of individuals belonging to each species that roost solitarily or in groups (SSA and MSA); (b) the natural variation in the number of individuals in SSA and MSA; (c) the species composition in MSA; (d) the patterns of species co-occurrence in MSA; and (e) the temporal variation in aggregation size and species composition in MSA. Our second goal is to examine one potential benefit and one potential cost of gregariousness in the seven *Prionostemma* species studied here, comparing the results between SSA and MSA. We tested the following hypotheses:

- If aggregations provide defense benefits, individuals roosting in aggregations would be in a safer condition than individuals roosting solitarily. Assuming that leg autotomy increases vulnerability to future predatory attacks and changes the perception of predation risk (Fleming et al., 2007; Emberts et al., 2019), we predict that autotomized individuals would be more prone to roost in groups because they would be more protected. Therefore, the probability of finding autotomized individuals roosting in aggregations would be higher than roosting solitarily. We also tested whether this probability differs between the types of aggregation. SSA and MSA confer several similar defense benefits, but at least one of these benefits is expected to be higher in MSA, the confusion effect (**Table 2**). Considering that the seven *Prionostemma* species show marked differences in color, and these differences may disrupt the search image of visually oriented predators, autotomized individuals could derive more defense benefits by joining MSA. Thus, the probability of finding autotomized individuals roosting in MSA would be higher than in SSA. Finally, assuming that most defense benefits of gregariousness are expected to be positively related to aggregation size, we predict that autotomized individuals would seek larger aggregations, where they would be safer.

- Given the close proximity of individuals, aggregations could increase the chance of horizontal transmission of ectoparasites. Therefore, we predict that individuals found in aggregations would be more likely parasitized by mites than solitary individuals. If the erythraeid mites that parasitize the *Prionostemma* species studied here have no host specificity, we expect similar costs of roosting in SSA and MSA. However, if the erythraeid mites have some level of host specificity, we expect the costs of roosting in SSA would be higher than in MSA.

Methods

Study Site

The research was conducted in Las Cruces Biological Station, Puntarenas, Costa Rica (8°47′ N; 82°57′W; 1,200 m. a.s.l.). The temperature in the study site ranges from 17 to 24°C, and the annual precipitation is about 3,600 mm, with a well-marked seasonality: a wet season between July and October, and a dry season between February and June. Data were collected in the understory of primary and secondary forests along the Jungle, Java, and Water trails.

Field Methods

We searched for *Prionostemma* harvestmen on three sampling occasions: January 2013, July 2013, and February 2014. Although the individuals were not marked, it is unlikely that they were resampled in different sampling occasions because Eupnoi harvestmen live only a few months as adults (Gnaspini, 2007). Moreover, we searched the trails only once and each individual and aggregation was sampled only once in the three sampling occasions. In all sampling occasions we searched for harvestmen during the day (08:00 to 14:00 h), when individuals are roosting motionless. On both sides of the trails, we looked for *Prionostemma* individuals roosting on the low vegetation (from 0 up to 2–3 m). For each individual, we recorded the species, which can be easily recognized based on the dorsal coloration (**Figure 3**), and the roosting status: (a) solitary, (b) in SSA, or (c) in MSA. For the purpose of this study, we defined aggregation as a group of two or more individuals, regardless of the species, resting in close proximity (<5 cm), with or without leg contact (**Figure 1D**). When we found isolated or aggregated individuals, we grabbed them by hand and quickly placed them inside plastic containers (30 cm diameter × 12 cm height). This allowed us to process one individual at a time while the others were caged in low density (2–4 individuals per container). After collecting the individuals, we carefully inspected each of them to record the number of missing legs, as well as the presence and number of mites on the body (including legs, dorsum, and venter).

We used the data collected in the three sampling occasions to describe the frequency of individuals belonging to each species that roost solitarily or in aggregations (SSA or MSA), the natural variation in the number of individuals in SSA and MSA, the species composition in MSA, and the patterns of species co-occurrence in MSA. Moreover, we used that data to test our hypotheses on benefits and costs of group living in harvestmen (see sections “Data analyses: benefits of gregariousness” and “Data analyses: cost of gregariousness” below).

In February-March 2014, we delimited a plot 1,100 m long and 6 m wide (3 m on each side of the above-mentioned trails). Inside this plot, we found 46 plants regularly used as roosting sites by *Prionostemma* individuals. These roosting sites were individually marked with numbered flags and inspected once every day (between 08:00 and 14:00 h) over 10.8 ± 1.3 days (range: 9–14 days). During each inspection, we recorded the number of individuals of each species in the roosting sites. We only used these data to describe the temporal variation in aggregation size and species composition.

Data Analyses: Benefits of Gregariousness

To test our predictions on the defense benefits of gregariousness, we ran generalized linear models (GLMs) in which *leg loss* (yes or no) was the predictor variable with multinomial distribution of errors and logit link function. The response variable was the roosting status with three levels: *solitary*, *SSA*, and *MSA*. This analysis was performed for each species separately, so that we could evaluate if the potential benefits of gregariousness are similar between them. Given that the number of individuals of *Prionostemma* sp.9, sp.10, and sp.11 was very low (15, 9, and 5, respectively), we could not perform the analysis for these species. Moreover, for *Prionostemma* sp.8 only MSA were found in the field, so that the response variable had only two levels (solitary and MSA). In this case, the GLM had a quasibinomial distribution of errors (to deal with data overdispersion) and the link function was logit. Based on the model for each species, we estimated the marginal means to calculate the necessary contrasts to test our predictions. We also ran models in which the predictor variable was the *number of missing legs* (ranging from 0 to 4). Given that the explanatory power of this model was similar to that of the leg loss model (**Supplementary Table 1**), we present here only the results obtained with the leg loss model.

Finally, we evaluated whether the *number of individuals* in the aggregations (i.e., aggregation size) and the *type of aggregation* (SSA or MSA) influenced the *proportion of autotomized individuals* in the aggregations. For this, we ran a GLM in which the response variable (i.e., the proportion of autotomized individuals in the aggregation) had a quasibinomial distribution of errors (to deal with data overdispersion) with logit link function. The full model included an interaction between aggregation size and type of aggregation.

Data Analyses: Cost of Gregariousness

To test our predictions on the costs of gregariousness, we ran two GLMs. In the first model, the response variable was the *presence of mites* (yes or no), with quasibinomial distribution of errors (to deal with data overdispersion) and logit link function. In the second model, the response variable was the *number of mites per individual*, with negative binomial distribution of errors (to deal with overdispersion of the data) and log link function. In both models, the predictor variable was the roosting status with three levels: *solitary*, *SSA*, and *MSA*. These analyses were performed for each species separately, so that we could evaluate if the potential costs of gregariousness are similar between them. Again, we excluded *Prionostemma* sp.9, sp.10, and sp.11 from the analyses because the number of individuals was very low. Based on the models for *Prionostemma* sp.5, sp.6, sp.7, and sp.8, we estimated the marginal means to calculate the necessary contrasts to test our predictions.

All statistical analyses were performed in the software R version 4.1.0 (R Core Team, 2021). We used the package *nnet* (Ripley et al., 2016) for the multinomial models, the package *Stats* (R Core Team, 2021) for the binomial models, the package *MASS* for the GLM with negative binomial distribution of errors (Venables and Ripley, 2002), and the package *emmeans* (Lenth, 2019) to calculate the contrasts. The complete datasets and the scripts used in the analyses are available on Dryad: https://datadryad.org/stash/share/TMMKprXz1Iji8hvzbumzaVDQi0g0jqxzLPfdl2btxpA.

Results

Gregariousness in *Prionostemma* Harvestmen

Taking together the three sampling occasions, we found 390 *Prionostemma* individuals, from which 73 (18.7%) were roosting solitarily and 317 (81.3%) in aggregations. The total number of aggregations was 78, being 56 (71.8%) MSA and 22 (28.2%) SSA. The aggregation size ranged from 2 to 9 in MSA and from 2 to 15 in SSA (**Figure 4A**). The number of species in MSA ranged from 2 to 5, with 92.3% of the aggregations containing 2 or 3 species.

The number of individuals was not evenly distributed among the seven *Prionostemma* species (Chi-square goodness-of-fit: $X^2 = 419.8$, df = 6, $P < 0.001$, **Figure 4B**). The two most common species were *Prionostemma* sp.5 (42.2% of all individuals) and *Prionostemma* sp.6 (31.1%); the other five species comprised together 26.7% of all individuals (**Figure 4B**). The species differed in the proportion of individuals that were found roosting solitarily or in aggregations (Chi-square goodness-of-fit: $X^2 = 23.6$, df = 6, $P < 0.001$, **Figure 4B**). For almost all species, individuals were more frequently found in aggregations than solitarily (**Figure 4B**). The only exception was *Prionostemma* sp.11, for which we collected only five individuals, one aggregated and four solitary (**Figure 4B**). When roosting in aggregations, individuals of *Prionostemma* sp.8, sp. 10, and sp. 11 were found only in MSA. In contrast, individuals of *Prionostemma* sp.5, sp.6, sp.7, and sp.9 were found both in MSA and SSA (**Figure 4B**).

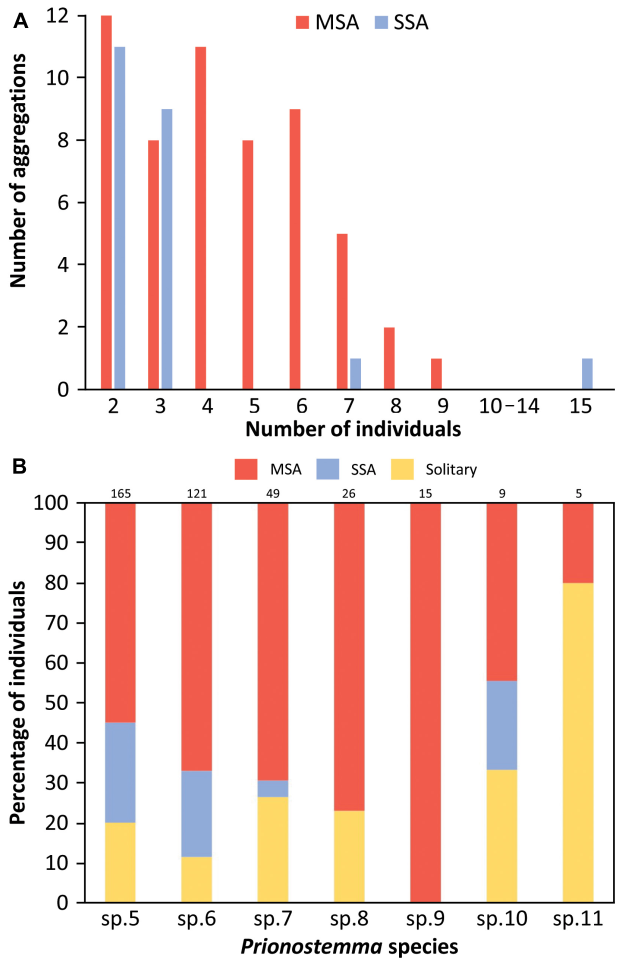


FIGURE 4 | (A) Number of individuals (i.e., aggregation size) in single-species aggregations (SSA) and mixed-species aggregations (MSA) of seven species of *Prionostemma* harvestmen from Costa Rica. **(B)** Relative frequency of individuals of each *Prionostemma* species found in the field roosting solitarily or in aggregations (SSA or MSA). Numbers above the bars indicate the total number of individuals of each species.

To explore the co-occurrence of species in MSA we constructed an association matrix. This matrix quantified the number and percentage of times each species was found together with the other species in the same aggregation (**Table 4**). The three most common species (*Prionostemma* sp.5, sp.6, and sp.7) were commonly found with each other (**Table 4**). Each of the four other species (*Prionostemma* sp.8, sp.9, sp.10, and sp.11) were associated in roughly similar proportions with all species (**Table 4**). Additionally, three species combinations represented 54% ($n = 30$) of all species combinations observed in the field: (a) *Prionostemma* sp.5 + sp.6 (23%), (b) *Prionostemma* sp.5 + sp.6 + sp.7 (23%), and (c) *Prionostemma* sp.5 + sp. 7 (7%).

The number of individuals in the roosting sites was highly variable over time, ranging from 1 to 16. The mean (±SD)

TABLE 4 | Matrix of paired associations of all seven *Prionostemma* species that form mixed-species aggregations (MSA).

***Prionostemma* species**	***Prionostemma* species**						
	sp.5	**sp.6**	**sp.7**	**sp.8**	**sp.9**	**sp.10**	**sp.11**
sp.5	—	34 (50%)	24 (46%)	9 (33%)	9 (35%)	3 (50%)	0 (0%)
sp.6	34 (43%)	—	17 (33%)	9 (33%)	7 (27%)	0 (0%)	1 (17%)
sp.7	24 (30%)	17 (25%)	—	4 (15%)	5 (19%)	2 (33%)	0 (0%)
sp.8	9 (11%)	9 (13%)	4 (8%)	—	4 (15%)	0 (0%)	1 (17%)
sp.9	9 (11%)	7 (10%)	5 (10%)	4 (15%)	—	1 (17%)	0 (0%)
sp.10	3 (4%)	0 (0%)	2 (4%)	0 (0%)	1 (4%)	—	4 (67%)
sp.11	0 (0%)	1 (1%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)	—
Total	79	68	52	77	26	6	6

The numbers above and below the diagonal are the same, as they represent the number of aggregations where at least one individual of a given species was found together with at least one individual of other species. The percentages in parentheses represent the frequency of each paired association in relation to the total number of MSA where each species was found ('Total' in the bottom line). Given that the total number of MSA differed between species and the percentages were always calculated taking the columns as a reference, the values in parentheses above and below the diagonal are not the same.

number of individuals was 3.3 ± 2.6 (n = 46), and the mean (±SD) coefficient of variation (CV) of the number of individuals across inspections was 55.2 ± 15.8% (range: 0–100%). The number of species in the roosting sites was also highly variable over time, ranging from 1 to 5. The mean (±SD) number of species was 1.8 ± 0.9 (*n* = 46), and the mean (±SD) CV across inspections was 43.3 ± 12.1% (range: 0–73.9%). Between two consecutive inspections, we recorded frequent transitions between all three categories of roosting status (**Figure 5**). The most frequent transition was from MSA to MSA, followed by MSA to solitary, and solitary to solitary (**Figure 5**). The least frequent transition was from solitary to SSA, followed by SSA to solitary, and MSA to SSA (**Figure 5**).

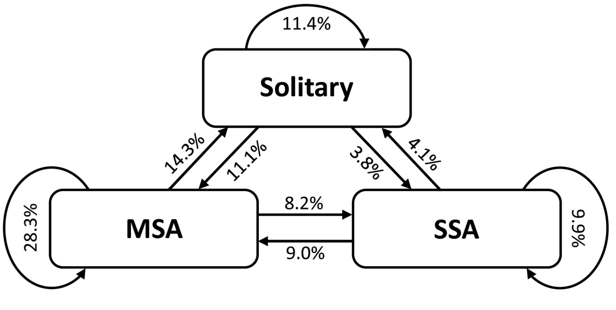


FIGURE 5 | Transitions between three categories of roosting status: solitary, single-species aggregations (SSA), and mixed-species aggregations (MSA). Data are based on daily inspections of 46 roosting sites used by *Prionostemma* harvestmen.

Benefits of Gregariousness

We recorded leg loss for 390 *Prionostemma* individuals. A total of 194 individuals (all species together) were found missing at least one leg. Most of the autotomized individuals in all seven species were missing only one (63.9%) or two legs (25.3%), but some of them were missing three (8.3%) or even four legs (0.5%); for four individuals (2.1%) we do not have information on the number of missing legs. The frequency of autotomized individuals differed between species (Chi-square goodness-of-fit: $X^2 = 232.7$, df = 6, $P < 0.001$), but the percentage of individuals missing at least one leg was always higher than 34% (**Figure 6A**).

Leg loss was not associated with the roosting status of the individuals in any of the four species analyzed (i.e., *Prionostemma* sp.5, sp.6, sp.7, and sp.8). In *Prionostemma* sp.5, sp.6, and sp.7, individuals were more likely found in MSA than in SSA or solitary, but this pattern did not differ between intact (i.e., eight-legged) and autotomized individuals (**Figures 7A–C**, see also contrasts 1–3 in **Figure 7E**). In *Prionostemma* sp.8, individuals were more likely found solitary than in MSA, but again this pattern did not differ between intact and autotomized individuals (**Figure 7D**, see also contrasts 1-3 in **Figure 7E**).

For *Prionostemma* sp.5 and sp.6, the probability of finding *intact* individuals in MSA was higher than finding *intact* individuals solitarily (**Figure 7**, contrast 5 in **Figure 7E**). Moreover, for *Prionostemma* sp.6 and sp.7, the probability of finding *intact* individuals in MSA was higher than in SSA (**Figure 7**, contrast 6 in **Figure 7E**). For *Prionostemma* sp.5, sp.6, and sp.8, the probability of finding *autotomized* individuals solitary, in SSA, and in MSA was similar (**Figure 7**, contrasts 7–8 in **Figure 7E**). For *Prionostemma* sp.7, however, the probability of finding *autotomized* individuals was higher in MSA than in SSA (**Figure 7**, contrasts 9 in **Figure 7E**). Lastly, the proportion of autotomized individuals in the aggregations was not associated with aggregation size or type of aggregation (**Table 5**).

Costs of Gregariousness

We recorded the presence of parasitic mites for 390 *Prionostemma* individuals. A total of 115 individuals (all species together) had mites, and for 111 we have information on the number of mites they were carrying. Mites were present in different body parts (legs, dorsum, and venter), either motionless (probably sucking on the host's hemolymph) or moving around (*n* = 12 observations). In 69.6% of all aggregations there was at least one individual carrying mites. The number of mites per individual ranged from 1 to 8, with the following relative frequencies: 1 = 57.7%, 2 = 25.2%, 3 = 9.9%, 4 = 2.7%, 5 = 2.7%, 6 = 0.9%, and 8 = 0.9%. The frequency of individuals carrying mites differed between species (Chi-square goodness-of-fit:

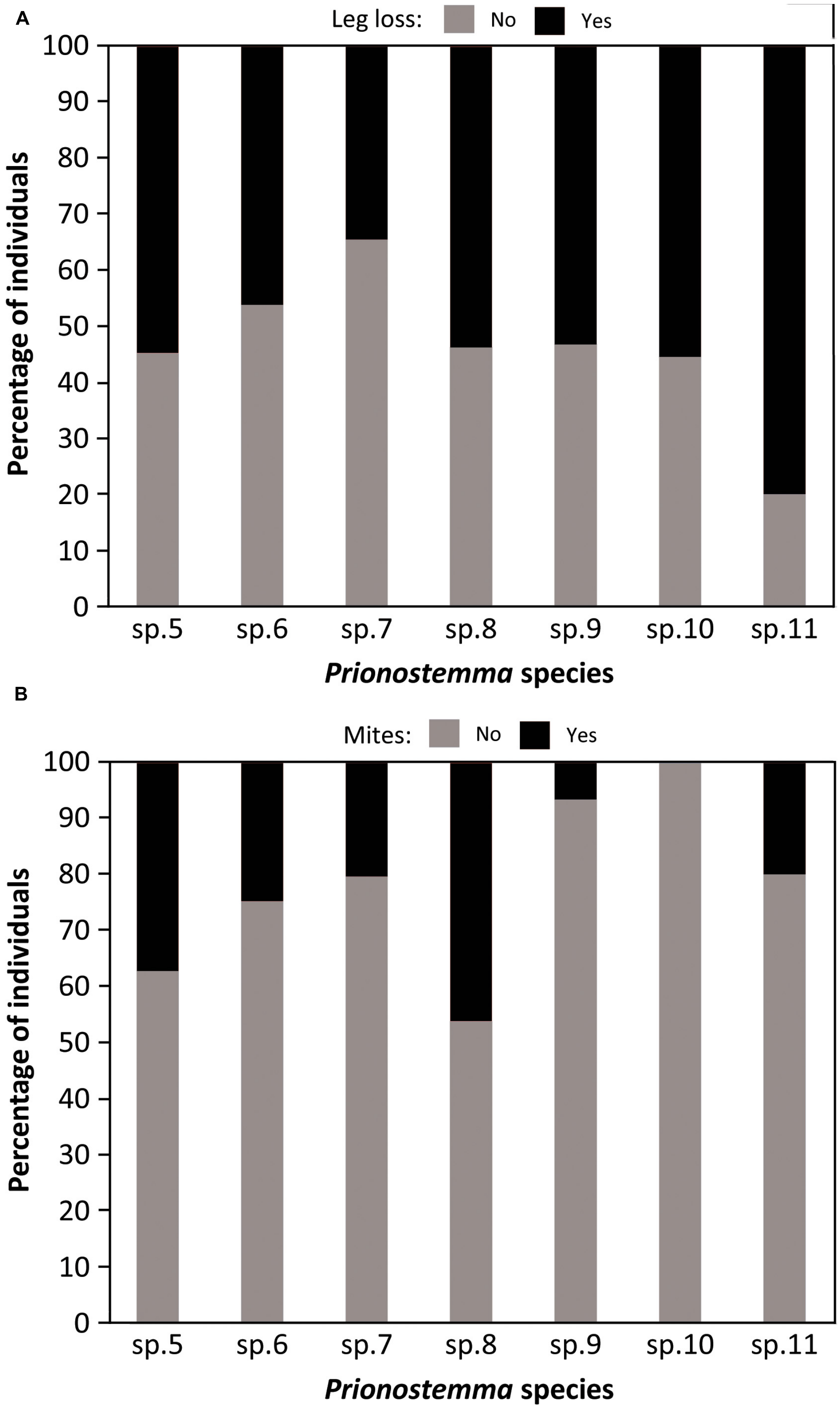


FIGURE 6 | (A) Relative frequency of leg loss among individuals of seven *Prionostemma* species. **(B)** Relative frequency of individuals carrying at least one mite among seven *Prionostemma* species. Sample sizes for each species are the same presented in **Figure 4B**.

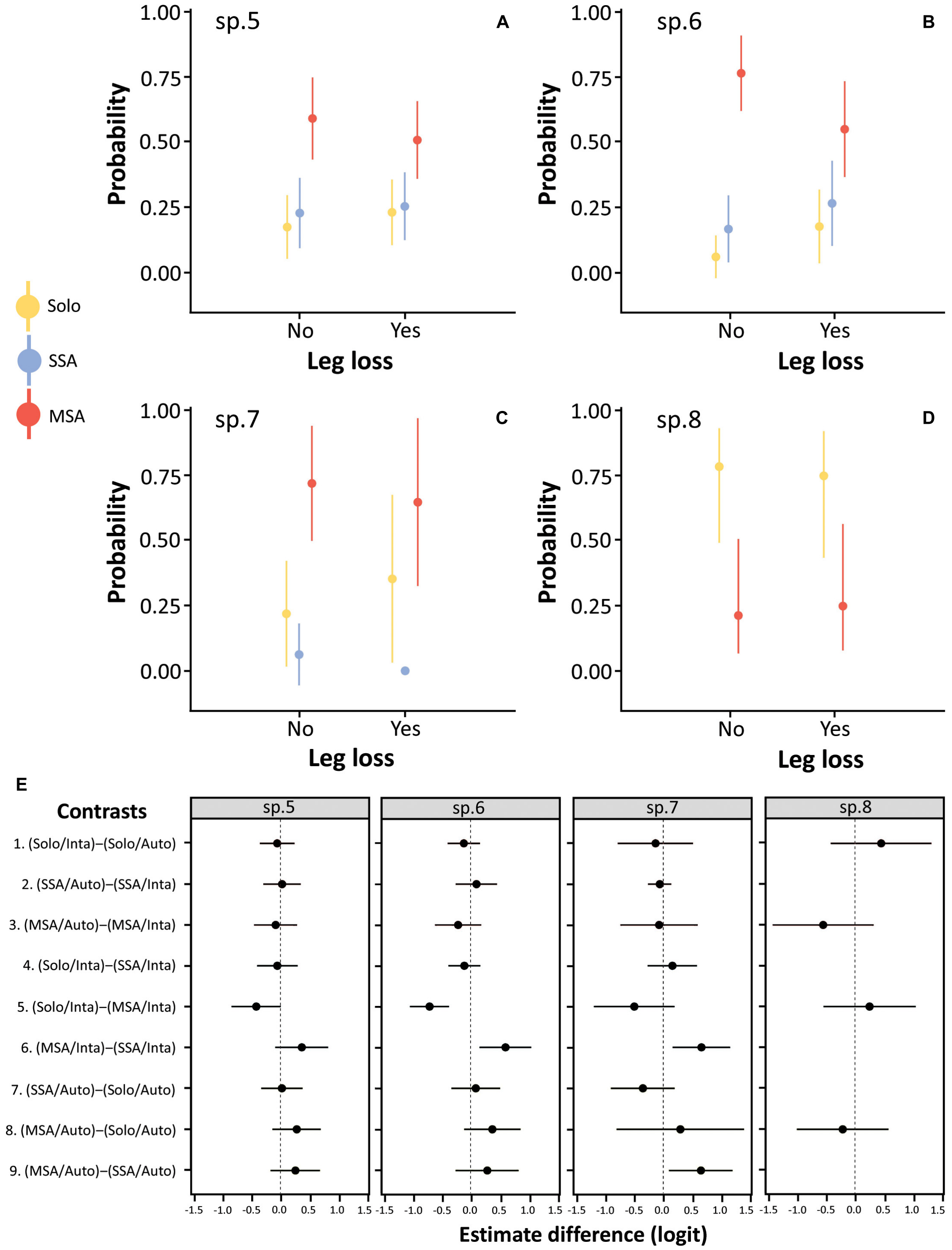


FIGURE 7 | The probability of finding individuals of four harvestman species roosting solitary, in single-species aggregations (SSA), or in mixed-species aggregations (MSA) as a function of leg loss. **(A)** *Prionostemma* sp.5, **(B)** *Prionostemma* sp.6, **(C)** *Prionostemma* sp.7, and **(D)** *Prionostemma* sp.8 (without records of SSA). **(E)** Contrasts of the probabilities for combinations of roosting status (Solo, solitary; SSA and MSA) and leg loss (Inta, intact individuals and Auto, autotomized individuals). Each contrast is calculated as the probability value of the combination within the first parentheses minus the probability value of the combination within the second parentheses. Thus, a positive value indicates that the estimated probability for the combination within the first parentheses is higher than the combination within the second parentheses, whereas a negative value indicates the opposite. To facilitate visual interpretation, we arranged the parentheses so that positive values support our predictions, i.e., autotomized individuals will have a higher probability of being found in aggregations, especially in MSA, where they would be more protected from predators. Circles indicate mean values **(A–D)** or mean differences between probabilities **(E)**. In all graphics, bars indicate 95% confidence intervals. In **(E)**, contrast values with 95% confidence interval overlapping 0 were considered as non-significant.

TABLE 5 | Results of the models to investigate the effect of type of aggregation (SSA, single-species; MSA, mixed-species) and aggregation size on the percentage of autotomized individuals of *Prionostemma* harvestmen.

Coefficients	Estimate	*SE*	*t*-value	*p*-Value
Intercept	–0.97	0.21	–4.532	<0.001
Aggregation size	0.03	0.04	0.762	0.449
Type of aggregation (SSA)	0.31	0.28	1.099	0.275
Aggregation size * Type of aggregation (SSA)	–0.01	0.05	–0.326	0.745

SE, standard error. The asterisk indicates statistical interaction between variables.

$X^2 = 181.3$, df = 6, $P < 0.001$, **Figure 6B**). However, for the four species we analyzed (i.e., *Prionostemma* sp.5, sp.6, sp.7, and sp.8), the roosting status had no effect on the probability of having mites (**Figure 8**) or the number of mites per individual (**Figure 9**).

Discussion

The Costa Rican *Prionostemma* is an interesting study system to understand gregariousness in harvestmen because the individuals of each species are facultatively gregarious, and can be found solitarily or in aggregations, forming both SSA and MSA. Thus, we could evaluate one potential benefit and one potential cost of gregariousness and compare them between SSA and MSA. In what follows, we first explore the descriptive information we gathered in the field and then we discuss our findings on benefits and costs.

Gregariousness in *Prionostemma* Harvestmen

When compared with other species of Eupnoi, the *Prionostemma* aggregations described here are small, with no more than 16 individuals. Among sclerosomatids from temperate regions (e.g., *Gyas*, *Leiobunum*, and *Nelima*), aggregations are composed of hundreds to thousands of individuals, usually packed in high density (e.g., Wagner, 1954; Holmberg et al., 1984; Novak et al., 2004). One possible reason for this difference in the number and density of individuals may be related to the habitat: while *Prionostemma* species inhabit tropical forests, temperate sclerosomatid species inhabit cold and/or xeric places (Machado and Macías-Ordóñez, 2007). In tropical forests, where temperature is warm and pluviosity is high, aggregations may have no relevant role in protecting the individuals against harsh abiotic conditions. In turn, in cold and/or xeric places, a high number of individuals packed together may confer physiological benefits, buffering stressful abiotic conditions (**Table 2**; see also Danks, 2002).

The *Prionostemma* aggregations described here show the largest number of species among all MSA reported so far for harvestmen (**Table 1**), despite being relatively small aggregations compared to other harvestman species. Additionally, for six of the seven species we studied, individuals were found mainly aggregated (instead of solitary), and more frequently in MSA than in SSA. These findings suggest that *Prionostemma* harvestmen are mainly gregarious and highly tolerant to heterospecifics. How and why individuals from different species are brought together remains to be explored. Harvestmen use pheromones for different purposes (Raspotnig, 2012), and there is evidence for one species of *Prionostemma* from Nicaragua that individuals mark roosting sites with chemical compounds (which might function as pheromones) and come back to those roosting sites (Donaldson and Grether, 2007; Grether et al., 2014b). However, the attraction of heterospecifics to MSA requires that the compounds present in the chemical signal (i.e., infochemicals) to be recognized by all aggregating species. It is currently unknown if all the *Prionostemma* species studied here produce and deposit recruitment infochemicals. Perhaps *Prionostemma* sp.5 and sp.6 – the two most common species in the study site – may act as 'initiators' of the MSA depositing recruitment pheromones. The other species, which are rarer, may act as 'followers' and join the MSA by eavesdropping on the infochemical. Although speculative, a similar mechanism has already been reported for the formation of mixed-species flocks in birds by means of differential production of vocalizations (Sridhar et al., 2009; Magrath et al., 2015; Goodale et al., 2019, 2020). Future work on *Prionostemma* can provide novel insights on the function of chemical compounds in harvestman, as well as the role of chemical communication in group formation.

Three combinations of species in MSA represented almost 54% of the field records (**Table 4**). This is surprising as seven species can generate 120 possible combinations of species. Additionally, the three most common species tended to occur together. For visually oriented predators, two of these common species may be regarded as cryptic (*Prionostemma* sp.6: dark gray body; and *Prionostemma* sp. 7: green body), whereas one of them may be regarded as conspicuous (*Prionostemma* sp.5: red body) (**Figure 3**). In all three most frequent associations, there was a mixture of a conspicuous species and at least one cryptic species. This difference in conspicuity may promote asymmetries in the defense benefits obtained by individuals of each species. Two possible scenarios of these between-species asymmetries are plausible. First, individuals of conspicuous species may be safer in MSA composed mainly of individuals of cryptic species, because SSA of conspicuous species may attract more attention of visually oriented predators than SSA of cryptic species (Vulinec, 1990). Second, assuming that all *Prionostemma* are chemically defended, individuals of cryptic species may be safer in MSA composed mainly of individuals of conspicuous species if predators avoid attacking aggregations of aposematic prey (e.g., Rippi et al., 2001). Alternatively, individuals of both cryptic and conspicuous species may enjoy similarly higher defense benefits in MSA if the confusion effect they cause on potential predators is more intense than in SSA (see section 'Benefits of mixed-species aggregations in harvestmen' below).

The number of individuals and species in the roosting sites showed marked variation over time. All possible transitions between the categories of roosting status (solitary, SSA, and MSA) were frequently recorded (**Figure 5**). To our knowledge, this is the first time that the temporal dynamic of mixed-species aggregations is explored in harvestmen. Our data reinforces previous suggestions that *Prionostemma* individuals are constantly moving between aggregations (Donaldson and Grether, 2007). Why individuals do that is an open question that deserves investigation. One possibility is that suitable roosting sites are very common in the study site. After leaving a roosting

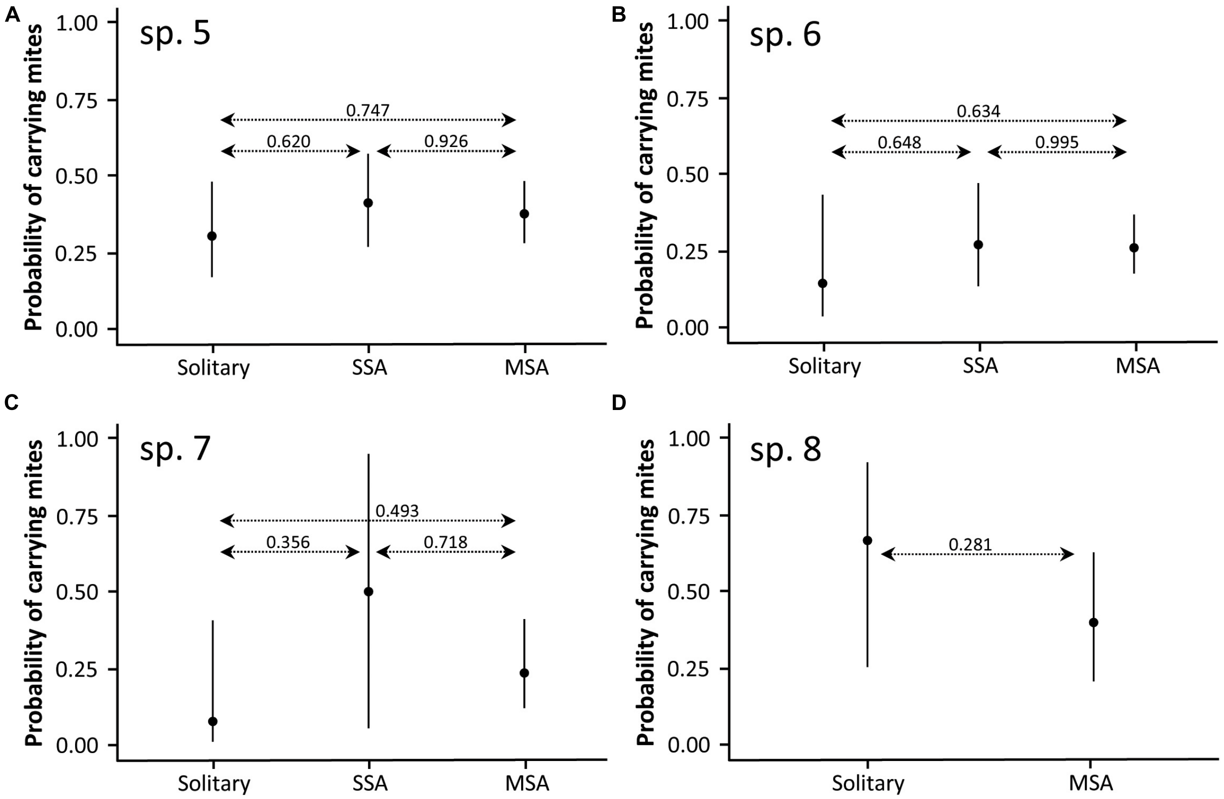


FIGURE 8 | Probability of finding individuals of four harvestman species carrying parasitic mites in response to their roosting status (solitary;SA, single-species aggregation; MSA, mixed-species aggregation). **(A)** *Prionostemma* sp.5, **(B)** *Prionostemma* sp.6, **(C)** *Prionostemma* sp.7, and **(D)** *Prionostemma* sp.8 (without records of SSA). Dashed lines indicate pairwise *P*-values. Circles indicate mean values and bars indicate 95% confidence intervals. Values of all contrasts are presented in **Supplementary Table 2**.

site at dusk, an individual may go far away searching for food or mating partners. At dawn, instead of paying the costs of returning to the original roosting site, these individuals may simply go to the best roosting site nearby. An important implication of the constant movement of individuals is that one roosting site may have an aggregation in 1 day and a solitary individual in the next day. Moreover, frequent transitions between SSA and MSA suggest that the benefits and costs associated with these two types of aggregations are similar. Otherwise, we would expect higher temporal stability and low frequency of transitions.

Benefits of Mixed-Species Aggregations in Harvestmen

We found a high frequency of leg loss in all *Prionostemma* species studied here (**Figure 6A**). This is consistent with previous reports for many Eupnoi harvestmen (e.g., Guffey, 1999; Houghton et al., 2011; Escalante et al., 2013, 2020, 2021; Domínguez et al., 2016; Escalante and Elias, 2021; Powell et al., 2021a,b). However, leg loss had no effect on the roosting status of the individuals, i.e., whether they were found solitary or in aggregations (SSA or MSA). Our hypothesis relating leg loss to the defense benefits of gregariousness assumed that autotomized individuals are more exposed to predation than intact individuals. Therefore, a first explanation for the lack of relationship between leg loss and roosting status is that our assumption does not hold. Although leg loss hampers the locomotor performance of sclerosomatid harvestmen (Escalante et al., 2013, 2020, 2021; Domínguez et al., 2016), a recent study showed that the recapture rates of autotomized and intact individuals of *Prionostemma* sp.5 are similar, suggesting that autotomy does not negatively impact individual survival (Escalante and Elias, 2021). That study also showed that autotomy changed micro-habitat use so that individuals missing legs were more frequently recaptured roosting among moss than on exposed tree trunks (Escalante and Elias, 2021). This finding suggests that autotomized individuals seek protection in concealed micro-habitats and perhaps this behavior is more effective in preventing predation than joining an aggregation.

If autotomized individuals are indeed more exposed to predation and find protection against predators in aggregations, we hypothesized that MSA would confer higher defense benefits due to the confusion effect, and predicted the probability of finding autotomized individuals roosting in MSA would be higher than in SSA. Our results support this prediction only for *Prionostemma* sp.7: the percentage of autotomized individuals was higher in MSA than in SSA. For *Prionostemma* sp.5 and sp.6, although the results we found are in the expected direction (i.e., a positive value of contrast 9 in **Figure 7E**), they were not

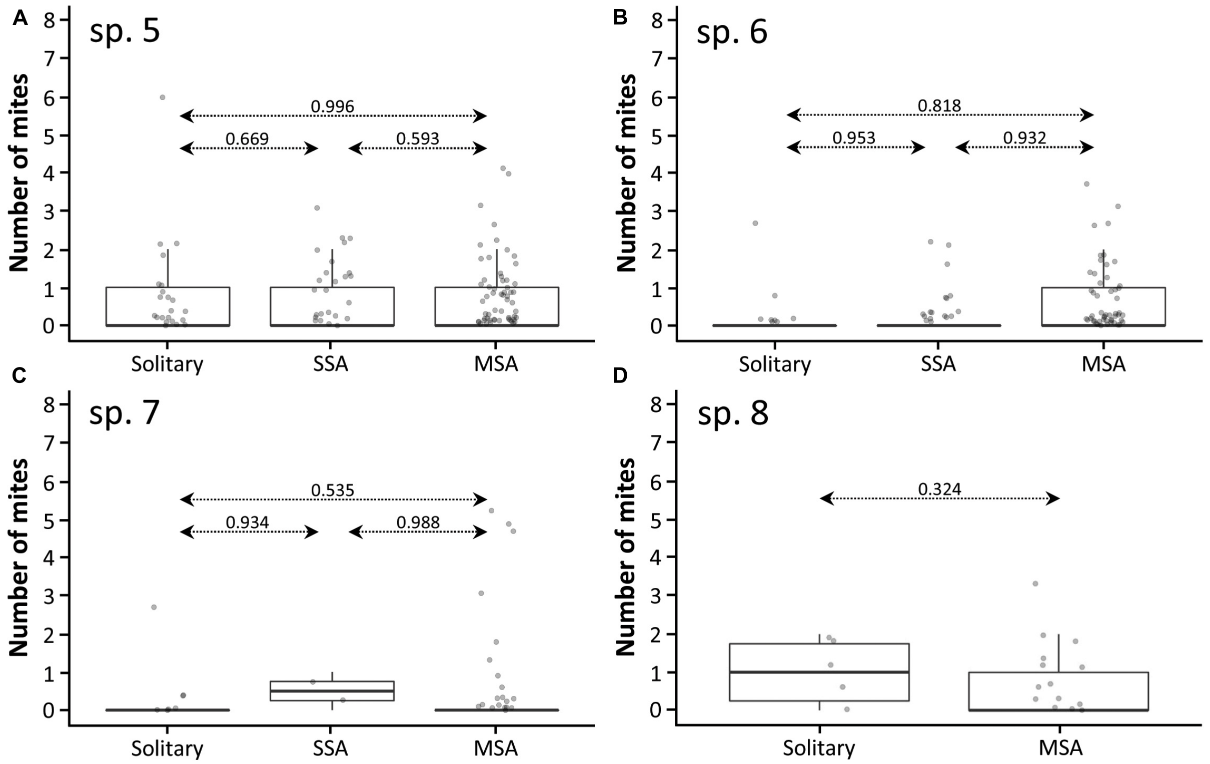


FIGURE 9 | Box-plots showing the number of mites carried by individuals in response to their roosting status (solitary; SSA, single-species aggregation; MSA, mixed-species aggregation) of four harvestman species. **(A)** *Prionostemma* sp.5, **(B)** *Prionostemma* sp.6, **(C)** *Prionostemma* sp.7, and **(D)** *Prionostemma* sp.8 (without records of SSA). Dashed lines indicate pairwise *P*-values. Values of all contrasts are presented in **Supplementary Table 3**.

statistically significant. This finding suggests that any possible defense benefit caused by the presence of multiple species in the aggregation is asymmetric. Whereas individuals of *Prionostemma* sp.7 may have higher defense benefits in joining MSA, the same is probably not true for *Prionostemma* sp.5 and sp.6. The reasons for this asymmetry remain to be better understood, but are unlikely to be explained by the confusion effect. As we mentioned above, *Prionostemma* sp.5, sp.6, and sp. 7 are commonly found together and show marked difference in body color. Thus, if the confusion effect emerges because phenotypic variation among aggregated species disrupts the search image of predators (**Table 2**), autotomized individuals of the three species should prefer to join MSA, which was not found in the field.

Finally, we expected that autotomized individuals of all species would prefer to join larger aggregations, where they would be more protected (Vulinec, 1990). This prediction was not supported by our data as aggregation size was not associated with the percentage of autotomized individuals. Although gregariousness may *decrease* the individual chance of predation via the dilution effect, it may also *increase* the chances of detection by predators because aggregations may be visually or chemically more conspicuous than isolated individuals (**Tables 2**, **3**). In sclerosomatid harvestmen, the main predators during daytime (when individuals are roosting) are probably birds (Cokendolpher and Mitov, 2007), which are visually oriented and may detect aggregations more easily than solitary individuals. Therefore, the encounter and dilution effects may operate in different directions (see **Figure 1** in Wrona and Dixon, 1991), and both need to be considered simultaneously in future studies (i.e., the attack-abatement effect *sensu* Turner and Pitcher, 1986) to fully assess the net fitness benefit of gregariousness.

Costs of Aggregating With Other Species

Mite prevalence for most *Prionostemma* species studied here (**Figure 6B**) is within the range already reported for other sclerosomatids worldwide, which varies from 16.7% (Townsend et al., 2008) to 61% (McAloon and Durden, 2000). In a comparative study with six harvestman species from Trinidad, Townsend et al. (2008) suggested that differences between species in mite prevalence are mainly related to habitat use: species that live or forage on the leaf litter are more likely to be parasitized because erythraeid mites lay their eggs in the soil. In a similar way, a study on two gregarious *Prionostemma* species in Nicaragua showed that mite prevalence varied between roosting sites (Grether et al., 2014a). In some spiny palms, no mites were found infesting the aggregated individuals, but in one of them, mite prevalence was 31.1%. Here, we investigated whether the roosting status of the individuals affects mite parasitism in the four most common *Prionostemma* species in our study site. We found that mite prevalence differed between species (**Figure 6B**), which suggests some level of host specificity or preference by the erythraeid mites. However, within species mite prevalence was similar for solitary and aggregated individuals – regardless of whether they were in SSA or MSA (**Figure 8**). This finding refutes our hypothesis that gregariousness increases the chance of horizontal transmission of ectoparasites.

The intensity of parasitism in the seven *Prionostemma* species studied here ranged from 1 to 8 mites per individual. As reported in previous studies with sclerosomatids from the United States (McAloon and Durden, 2000) and phalangiids from Bulgaria (Mitov, 2000), most individuals carried few mites, whereas a few individuals were heavily parasitized. Clumped parasite distributions on hosts are a widespread pattern in animals (Leung, 1998). In the case of erythraeid mites, only the larval stages are parasitic, so that the high parasite load of few *Prionostemma* individuals may result from a host being exposed to an aggregation of larvae just after they hatch (McAloon and Durden, 2000). Despite the great inter-individual variation, we showed that the intensity of parasitism was not affected by the roosting status (**Figure 9**). This finding reinforces the conclusion that aggregating with conspecific or heterospecifics does not bring different costs in terms of mite parasitism in *Prionostemma* harvestmen.

The fluid membership of the *Prionostemma* aggregations, with marked daily turnovers in the number of individuals and species composition at the roosting sites (**Figure 5**), can have important implications for the transmission of mites between individuals. Given that individuals are likely changing their roosting status over time, a snapshot sample (as the one conducted here) does not provide a precise picture of their risk of being parasitized. A recent mathematical model has shown that aggregation size and roost site fidelity are key factors influencing pathogen spreading in populations of gregarious species (Laughlin et al., 2019). According to this model, pathogens spread faster among roosting sites when (i) individuals are distributed among a large number of small aggregations and (ii) exhibit low roost site fidelity. These two conditions apply to *Prionostemma* harvestmen (**Figures 4A**, **5**), which may explain why nearly 70% of all aggregations had at least one individual carrying mites. However, to better understand the factors that influence the individual risk of being parasitized, future research should be conducted with individually marked harvestmen. The question to be explored is whether individuals with higher tendency of being found in aggregations are more likely to be parasitized than those with higher tendency of being found solitary. There is increasing evidence that individuals vary widely in their probability of contracting and spreading parasites (Barron et al., 2015), and *Prionostemma* harvestmen offer an opportunity to explore this subject in species that form both SSA and MSA.

CONCLUSION

Our review highlights that most of the potential benefits of MSA in harvestmen are similar to those reported for SSA in other taxa (**Figure 2**; see also Boulay et al., 2019; Goodale et al., 2020). For the physiological benefits, for instance, aggregations may reduce metabolic rates regardless of whether they are composed of one or multiple species. Most of the defense benefits — dilution effect, confusion effect, increased vigilance, and collective retaliation — should also work in SSA and MSA. However, there are circumstances in which the physiological and defense benefits in MSA are expected to be asymmetric, with some species obtaining more benefits than others (**Figure 2**). In harvestmen, an extreme example of this asymmetry probably occurs when only one species is chemically defended. In this case, individuals of the non-defended species may be regarded as parasites of both the alarm signal and the defensive compounds released by individuals of the chemically defended species (Machado and Vasconcelos, 1998). Lastly, in some circumstances, MSA may confer higher benefits than SSA. For instance, future studies should experimentally address the potential increase in the confusion effect in MSA, which should be higher than in SSA if variation in color between species disrupts the search image of predators even further.

Contrary to the benefits, our review suggests that most of the potential costs of MSA for harvestmen differ from those reported for SSA in other taxa (**Figure 2**; see also Goodale et al., 2020). For instance, if pathogens and parasites are species-specific, the likelihood of transmission may be lower when individuals are aggregated with heterospecifics — a pattern already reported for birds (e.g., Poulin, 2010; González et al., 2014). Moreover, if aggregated individuals fiercely compete for resources when they leave the aggregations, MSA may attenuate competition because interspecific variation is also expected in their diet and microhabitats used for foraging (e.g., Krasnov et al., 2006; but see Kaplan and Denno, 2007). Finally, if aggregations are related to reproduction, reproductive costs, such as sexual harassment, extra-pair copulation, misdirect parental care, and infanticide, should be lower in MSA than in SSA due to the lower density of conspecifics nearby (**Figure 2**). In all examples mentioned so far, the costs of MSA are lower than SSA, but there are two exceptions: the costs related to increased conspicuousness to predators and increased risk of pathogens and parasites transmission. In these two cases, if individuals of different species are equally conspicuous to predators and susceptible to non-specific parasites, the costs of MSA should be similar to those of SSA (**Figure 2**).

In our empirical study, we investigated one potential benefit and one potential cost of gregariousness in seven *Prionostemma* species that roost either solitarily or in groups, forming SSA or MSA. Although our data provide an observational snapshot of the study system, they are the first formal test of the hypotheses that gregariousness confers defense benefits but incurs costs in terms of increased parasitism. We found that intact and autotomized individuals of the four most common *Prionostemma* species have similar probability of being found solitarily or in aggregations. This result refutes our hypothesis that autotomized individuals would be found preferentially in aggregations, where they would be more protected from predators. We stress, however, that our findings do not discard the possibility that harvestman aggregations improve defense since our test does not directly address any of the specific defense benefits in **Table 2**. Regarding the costs, we found that mite prevalence or infestation intensity were similar between solitary or aggregated individuals. This result refutes our hypothesis that gregariousness would increase the chances of mite parasitism in harvestmen. We suggest that the fluid membership of the aggregations, with great variation over time in the number of individuals and species composition, may explain the lack of relationship between roosting status and mite parasitism.

Overall, we expect that both our review and the empirical results stimulate further investigation on group living in harvestmen. We highlighted many questions to be answered in fields of knowledge as diverse as physiology, chemical ecology, parasitology, and behavioral ecology. Moreover, the frequency of species that form MSA in harvestmen is higher than any other arthropod taxa (see Table 1 in Boulay et al., 2019 and **Table 1** in the present study). In the same species that form MSA, individuals are also found solitarily or forming SSA. As we showed here, the genus *Prionostemma* offers a unique opportunity to quantify the benefits and costs of gregariousness and to compare them between different types of aggregation.

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: Dryad (https://datadryad.org/stash/share/TMMKprXz1Iji8hvzbumzaVDQi0g0jqxzLPfdl2btxpA).

AUTHOR CONTRIBUTIONS

All authors contributed equally to the conceptualization, data curation, analysis, and review and editing of the manuscript. IE, MD, and DAG-R conducted the field-data collection. IE and GM led the writing of the original draft. IE led the funding acquisition and project administration.

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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.2021.766323/full#supplementary-material

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REVIEW
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Ecological Drivers of Non-kin Cooperation in the Hymenoptera

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Despite the prominence of kin selection as a framework for understanding the evolution of sociality, many animal groups are comprised of unrelated individuals. These non-kin systems provide valuable models that can illuminate drivers of social evolution beyond indirect fitness benefits. Within the Hymenoptera, whose highly related eusocial groups have long been cornerstones of kin selection theory, groups may form even when indirect fitness benefits for helpers are low or absent. These non-kin groups are widespread and abundant, yet have received relatively little attention. We review the diversity and organization of non-kin sociality across the Hymenoptera, particularly among the communal bees and polygynous ants and wasps. Further, we discuss common drivers of sociality across these groups, with a particular focus on ecological factors. Ecological contexts that favor non-kin sociality include those dominated by resource scarcity or competition, climatic stressors, predation and parasitism, and/or physiological constraints associated with reproduction and resource exploitation. Finally, we situate Hymenopteran non-kin sociality within a broader biological context by extending insights from these systems across diverse taxa, especially the social vertebrates. Non-kin social groups thus provide unique demonstrations of the importance of ecological factors in mediating the evolutionary transition from solitary to group living.

Keywords: social evolution, relatedness, kinship, wasps, bees, ants

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INTRODUCTION

Social animals represent some of the most ubiquitous and ecologically dominant organisms globally (Hölldobler and Wilson, 1990; Krause and Ruxton, 2002; Ward and Webster, 2016). To date, our understanding of how social groups emerge has been rooted overwhelmingly in the study of family groups. From these groups have emerged useful theoretical frameworks for explaining cooperation in nature, especially kin selection theory, which posits that indirect fitness benefits of helping kin can compensate for direct fitness costs (Hamilton, 1964; West-Eberhard, 1975; Trivers and Hare, 1976; Abbot et al., 2011; Bourke, 2014). Nevertheless, many animals form groups with non-relatives, and in these societies direct fitness gains are generally the major component of inclusive fitness (Clements and Stephens, 1995; Dugatkin, 2002; Goodnight, 2005; Clutton-Brock, 2009; Queller, 2011). These social groups, which exist across diverse animal taxa

(Bernasconi and Strassmann, 1999; Clutton-Brock, 2009; Riehl, 2013; Wilkinson et al., 2016; Brask et al., 2019; Suarez and Goodisman, 2021), demonstrate the value of examining the diversity of selection contexts for understanding the evolution of sociality, and provide useful models for examining ecological drivers of social evolution.

Kin selection has proven critically valuable for understanding the evolution of eusociality, especially within the highly related colonies of the social insects (West-Eberhard, 1975; Queller and Strassmann, 1998; Hughes et al., 2008; Abbot et al., 2011; Bourke, 2011; Linksvayer and Wade, 2011). However, eusociality is rare; even among the Hymenoptera; other forms of group living are considerably more common (Heinze et al., 2017; Hunt and Toth, 2017; Wcislo and Fewell, 2017; Fewell and Abbot, 2018). Perhaps due to the prominence of kin selection as a framework for understanding insect sociality, non-kin groups in insects have received relatively little attention, despite advances in our understanding of non-kin vertebrate groups (Clutton-Brock, 2009; Riehl, 2013; Wilkinson et al., 2016; Brask et al., 2019). Departures from a kin-centric framework for understanding insect social evolution may enable valuable connections to other animal groups, contributing to a broader body of evolutionary theory. Further, these systems may be neglected because interactions among non-kin rarely (if ever) constitute altruism—that is, behavior that reduces the fitness of the actor and increases the fitness of the group—which has been a major focus of social evolutionary research in the eusocial Hymenoptera (Hamilton, 1972; Simon, 1990; Foster et al., 2006; Kennedy et al., 2018). Rather, non-kin associations provide examples of cooperation based on mutual benefits of grouping, with or without reproductive division of labor.

We review advances in our understanding of non-kin social groups in the Hymenoptera, with a focus on patterns of diversity in social structure and ecological context. We characterize variation in the organization of these groups and describe the distribution of non-kin sociality across the bees, ants, and wasps. Across these groups, we then highlight common ecological drivers of non-kin sociality, particularly environmental challenges and intra- and inter-specific interactions. Finally, we synthesize insights from the current body of research on non-kin sociality and highlight promising directions for future research. In doing so, we emphasize the role of ecological context in shaping sociality at its evolutionary origins.

NON-KIN COOPERATION IN THE HYMENOPTERA

Non-kin sociality is found broadly among the social ants, wasps, and bees, and ranges in complexity from simple, facultative nest sharing in primarily solitary populations to cooperative founding of eusocial colonies (**Figure 1**). For the purposes of this review, we define sociality as any long-term association between conspecifics characterized by mutual tolerance and/or cooperation within shared nesting space (Costa, 2006; Fewell and Abbot, 2018). By "long-term," we refer to an extended or significant portion of an individual's lifespan, as opposed to more transient interactions like mating. Further, we emphasize mutual tolerance as a minimum requirement in our definition of sociality for the sake of including even groups characterized by limited cooperative behavior. Mutual tolerance serves as a preadaptation for the evolution of cooperation, by enabling individuals to share nest space and providing opportunities for more complex social interactions (Michener, 1974, 1990a).

Specifically, we examine social interactions in the context of breeding and offspring care, because behavioral decisions in these contexts have important fitness impacts. We emphasize nest sharing to exclude from our definition of sociality those animals living within aggregations of spatially clustered nests, but otherwise living solitarily. Though some Hymenoptera (such as army ants) are non-nesting, nests are used predominantly by this taxon as an essential physical site for the prolonged interactions intrinsic to social living. Additionally, we define sociality as distinct from intraspecific social parasitism, and therefore exclude from our discussion those systems in which non-kin relationships arise through parasitic behavior (Beekman and Oldroyd, 2008), including adoption of unrelated offspring (Klahn, 1988; Nonacs and Reeve, 1993) and cleptoparasitism (Michener, 1974; Rozen, 1991).

Non-kin associations vary considerably in the degree of cooperation, and thus serve as an important counterpoint to vertebrate sociality. However, discussions of cooperation for social insects and social vertebrates have historically been treated separately. For example, cooperation in the social insects is often studied in the context of task allocation and division of labor (Hölldobler and Wilson, 1990; Seeley, 1996; Beshers and Fewell, 2001), while social vertebrate sociality is more often discussed in terms of the costs and benefits of cooperative interactions (Hamilton, 1964; Dugatkin, 2002; Clutton-Brock, 2009). Defining cooperation itself has also presented challenges, with debate surrounding the questions of whether cooperative interactions may incur differential costs for actor and recipient, and whether cooperative sociality can be maintained under such conditions without indirect fitness gains (Lehmann and Keller, 2006; West et al., 2006, 2007; Bergmüller et al., 2009). Within such discussions, however, has emerged a central theme that cooperation broadly entails behaviors that benefit the social group (Clutton-Brock, 2009).

Social Evolution in the Hymenoptera

The evolution of cooperative behaviors is shaped by ecological context and by the phylogenetic pathway that group has taken to sociality. The task of categorizing the various forms of sociality and their evolutionary histories has been the subject of considerable debate (Wilson, 1971; Michener, 1974; Crespi and Yanega, 1995; Toth et al., 2016; Boomsma and Gawne, 2018; Richards, 2019). A well-established hypothesis has proposed a stepwise evolutionary progression from simple forms of sociality to complex eusociality (Evans, 1956; Wilson, 1971; Evans and West-Eberhard, 1973; Rehan and Toth, 2015). Recent, renewed discussion of this topic has challenged the theoretical presumption of a "social ladder" in which less complex social forms represent intermediate "levels" along an evolutionary trajectory toward eusociality (Linksvayer and Johnson, 2019;

FIGURE 1 | Examples of non-kin sociality are widespread across hymenopteran taxa. In the ants, unrelated foundresses may cooperate to rear eusocial colonies, as in the harvester ant *Pogonomyrmex californicus* (top left; photo by Elizabeth Cash). Similarly, foundresses of some wasp species, like the paper wasp *Polistes dominula* (bottom; photo by Meagan Simons), may cooperatively found eusocial nests with non-relatives. Non-kin associations are also found among the communal bees, such as the sweat bee *Agapostemon virescens* (top right; photo by Nicholas Dorian), which shares nest-entrance guarding duties with unrelated nestmates.

Holland and Bloch, 2020). Accordingly, we consider the diversity of cooperative systems in the social insects not as transitional forms in the evolution of sociality, but instead in terms of their shared cooperative behavioral repertoires that are adaptive in a given ecological context.

One of the simplest forms of sociality, known as communal living, refers to societies in which multiple same-generation females (often unrelated) share nesting space but independently forage and provision their own offspring (Michener, 1974). Communal groups are characteristically casteless: group members are not distinguished behaviorally or morphologically by their capacity for reproduction. Only a subset of tasks—typically nest construction and nest defense—are shared cooperatively. Communal groups often exist among otherwise solitary populations of bees and wasps, and are characterized by behavioral repertoires similar to those of solitary females: they mass-provision brood at the egg stage, and do not engage in further direct parental care (Wcislo and Tierney, 2009; Wcislo and Fewell, 2017). In contrast, other social insects, including ants, wasps, and some bee taxa, perform direct parental care in which larvae are fed progressively (Field, 2005). The cooperative repertoire of these groups is similarly expanded. These associations occur when related or unrelated females found nests cooperatively (pleometrosis) by sharing or dividing such tasks as provisioning, nest construction, and defense (Ross and Matthews, 1991; Heinze et al., 2017).

Social Diversity in the Hymenoptera

Here we describe the diversity of non-kin sociality defined as long-term adult nest sharing, with groups often characterized by cooperative behaviors and task sharing. Because relatedness is a relative attribute (Pamilo, 1989), we do not strictly define kin vs. non-kin, but rather focus on groups in which individuals may be no more related to their nestmates than they are to non-nestmates. For some of the systems we discuss, non-kinship in social groups has been evaluated with high confidence by inferring relatedness from molecular markers. In many other cases, the presence of non-relatives in social groups has been inferred from observations of nest-joining behavior, often by individuals from distant nests (in bees and wasps), or of cooperative nest founding by presumed unrelated foundresses (in wasps and ants). Though these observations cannot confirm the degree of relatedness between joiners and their nestmates, they provide suggestions of potential flexibility in tolerance toward unrelated conspecifics. Because the data on kinship in these groups is so incomplete, we highlight these uncertain cases as promising avenues for future genetic investigation.

Within the Hymenoptera, we explore non-kin groups among wasps, bees, and ants, finding limited evidence for true sociality among the sawflies (Hymenoptera: Symphyta), which have short adult lifespans and are non-nesting (Kudo et al., 1998). For each group, we describe patterns and diversity of non-kin social systems. We do not present an exhaustive review of all known non-kin groups in the Hymenoptera, but instead highlight common patterns of social organization across the major suborders.

Wasps: Communal Societies and Foundress Associations

The wasps (Hymenoptera: Apocrita) comprise more than 37 families, among which only three (Aculeata: Pompilidae, Sphecidae, and Vespidae) contain social species (Hunt and Toth, 2017). Non-kin groups are found within all three of these families (**Table 1**). Communal nesting has been described for several species, and among these, nest-joining by non-relatives is possible, though unconfirmed, for the spider wasp *Auplopus semialatus* (Pompilidae: Pepsinae); (Wcislo et al., 1988), the digger wasp *Crabro cribrellifer* (Crabronidae: Crabroninae); (Wcislo et al., 1985), and the pollen wasp *Trimeria howardii* (Vespidae: Masarinae); (Zucchi et al., 1976). Facultative nest sharing is likewise known among the hover wasps (Vespidae: Stenogastrinae), where unrelated females can join established foundresses (Strassmann et al., 1994; Turillazzi, 2012). Similarly, among the primitively eusocial paper wasps (Vespidae: Polistinae), foundress associations often form among sisters or other close relatives (West-Eberhard, 1969; Ross and Matthews, 1991), but in many cases may be comprised of non-kin (Queller et al., 2000; Hunt, 2007; Mora-Kepfer, 2014). For the paper wasp *Polistes dominula*, 15–35% of foundress associations consist of unrelated females (Queller et al., 2000; Zanette and Field, 2008; Leadbeater et al., 2011). Co-founding by non-relatives is also known, but uncommon, in *Polistes fuscatus* (Klahn, 1979) and *Polistes exclamans* (MacCormack, 1982). Unlike communal groups, these societies are characterized by high reproductive skew, so unrelated joiners often become subordinate helpers with limited reproductive opportunities (Queller et al., 2000; Leadbeater et al., 2010; Mora-Kepfer, 2014).

Bees: Communal and Parasocial Societies

Communal nesting occurs across all six major bee families (Wcislo, 1993; Kukuk et al., 2005), and many of these communal groups are known or expected to consist of non-kin. This social strategy is perhaps best known among the sweat bees (Halictidae), which are known for their incredible diversity of social behaviors (Michener, 1974, 1990b, 2007; Brady et al., 2006). Halictid communal nesting has been described within the subfamilies Halictinae and Nomiinae; for most of these species, relatedness among communal nestmates is unknown (Michener, 1969; Wcislo, 1993; Vogel and Kukuk, 1994; Wcislo and Engel, 1996), but may be inferred to be low through observations of nest-joining behavior (Michener and Lange, 1958; Abrams and Eickwort, 1981; Richards et al., 2003). Kukuk and Sage analyzed two polymorphic genetic loci among colonies of the sweat bee *Lasioglossum hemichalceum* (Halictidae: Halictinae) and found relatedness within reproductively active nests to be indistinguishable from zero (1994). Communal nesting is present but less common among the Colletid bees (Sakagami and Zucchi, 1978), with low relatedness (r = 0.26) confirmed among nestmates of *Amphylaeus morosus* (Colletidae: Hylaeinae) (Spessa et al., 2000). Similarly, non-kin nesting is possible among the communal Andrenidae (Danforth, 1991; Paxton et al., 1999), and has been confirmed for two species: *Andrena scotica* (formerly *jacobi*); (Andrenidae: Andreninae) (Paxton et al., 1996) and *Macrotera* (formerly *Perdita*) *texana* (Andrenidae: Panurginae) (Danforth et al., 1996).

In other cases, the social organization of some non-kin bee groups is more aptly described by the umbrella term "parasocial," which includes all associations of same-generation adults, which may be cooperative or non-cooperative, and which may exhibit high or low reproductive skew (Michener, 1974). This is the case for many bees of the family Apidae, which includes both solitary and highly social species. For example, bees in the genus *Exomalopsis* (Apidae: Apinae) form multi-female nests, which may be characterized by cooperative provisioning (Michener, 1966) and even reproductive skew (Raw, 1977). Relatedness in this genus has not been formally investigated, but is likely to be low for many species, considering the high number of females per nest (884 in one nest of *E. aureopilosa*; Rozen, 1984). Non-kin associations could also be found among pleometrotic foundresses of eusocial colonies, though this is rare within the bees. Low relatedness has been described for co-foundresses of the primitively eusocial sweat bee *Halictus ligatus*, likely arising from chance encounters among females emerging from their winter hibernacula (Richards and Packer, 1998).

An interesting case of non-kin sociality exists among the large carpenter bees in the genus *Xylocopa* (Apidae: Xylocopinae). Nest-joining behavior has been observed in several species, in many cases by unrelated bees (Gerling, 1982; Gerling et al., 1983; Velthuis, 1987; Michener, 1990a; Hogendoorn and Leys, 1993; Peso and Richards, 2011). However, low relatedness in social groups has only been demonstrated with molecular evidence for two species, *X. sonorina* and *X. virginica* (Ostwald et al., 2021a, this issue; Vickruck and Richards, 2021, this issue). Sociality in these groups is not easily classified, given variation and ambiguity in helping behavior, reproductive skew, and generational overlap (Gerling et al., 1989; Michener, 1990a; Hogendoorn and Velthuis, 1993). In most cases, a single dominant female per social nest will monopolize egg laying and provisioning behavior, with nestmates potentially contributing to nest guarding (Gerling et al., 1983, 1989; Hogendoorn and Velthuis, 1999; Buchmann and Minckley, 2019).

Ants: Foundress Associations and Primary Polygyny

In the ants, non-kin sociality through pleometrosis is relatively commonplace in incipient colonies, but usually ends with a queen culling event triggered by worker emergence (Bernasconi and Strassmann, 1999). However, permanent non-kin social groups

TABLE 1 | Hymenopteran species with the strongest evidence for non-kin associations.

	Taxon	Social organization	Evidence for non-kin sociality	Within-group *r*	References
Wasps	Vespidae				
	Stenogastrinae				
	Liostenogaster flavolineata	Primitively eusocial	Allozyme analysis	Not reported for foundresses	Strassmann et al., 1994
	Polistinae				
	Mischocyttarus mexicanus	Primitively eusocial	Behavioral observations	NA	Mora-Kepfer, 2014
	Polistes exclamans	Primitively eusocial	Behavioral observations	NA	MacCormack, 1982
	Polistes fuscatus	Primitively eusocial	Behavioral observations	NA	Klahn, 1979
	Polistes dominula	Primitively eusocial	Microsatellite analysis	∼0.1 (for 15% of population)	Queller et al., 2000; Zanette and Field, 2008
Bees	Andrenidae				
	Panurginae				
	Macrotera texana	Communal	DNA fingerprinting	0.008	Danforth et al., 1996
	Andreninae				
	Andrena scotica	Communal	Microsatellite analysis	∼0	Paxton et al., 1996
	Halictidae				
	Halictinae				
	Lasioglossum hemichalceum	Communal	Allozyme analysis	0.07	Kukuk and Sage, 1994
	Halictus sexcinctus	Communal or primitively eusocial	Behavioral observations	NA	Richards et al., 2003
	Halictus ligatus	Primitively eusocial	Allozyme analysis	−0.18	Richards and Packer, 1998
	Agapostemon virescens	Communal	Behavioral observations	NA	Abrams and Eickwort, 1981
	Pseudagapostemon divaricatus	Communal	Behavioral observations	NA	Michener and Lange, 1958
	Colletidae				
	Hylaeinae				
	Amphylaeus morosus	Communal	Allozyme analysis	0.26	Spessa et al., 2000
	Apidae				
	Xylocopinae				
	Xylocopa virginica	Parasocial	Microsatellite analysis	0.09–0.30	Vickruck and Richards, 2021, this issue
	Xylocopa sonorina	Parasocial	Microsatellite analysis	−0.09–0.35	Ostwald et al., 2021a, this issue
	Xylocopa sulcatipes	Parasocial or semisocial	Behavioral observations	NA	Velthuis, 1987
	Xylocopa pubescens	Parasocial or semisocial	Behavioral observations	NA	Gerling et al., 1983; Hogendoorn and Leys, 1993
Ants	Formicidae				
	Myrmecinae				
	Atta texana	Eusocial	Behavioral observations	NA	Moser and Lewis, 1981
	Acromyrmex versicolor	Eusocial	Allozyme analysis	−0.12	Rissing et al., 1989
	Acromyrmex heyeri	Eusocial	Isozyme analysis	Not reported	Diehl et al., 2001
	Acromyrmex striati	Eusocial	Isozyme analysis	Not reported	Diehl et al., 2001
	Myrmica gallienii	Eusocial	Isozyme analysis	0.01	Seppä, 1996
	Pogonomyrmex californicus	Eusocial	Microsatellite analysis	0.059	Overson et al., 2016

(Continued)

TABLE 1 | (Continued)

Taxon	Social organization	Evidence for non-kin sociality	Within-group *r*	References
Messor pergandei	Eusocial	Microsatellite analysis	∼ 0	Helms and Helms Cahan, 2012
Camponotus ligniperdus	Eusocial	Microsatellite analysis; DNA fingerprinting	Not reported	Gadau et al., 1998
Formicinae				
Myrmecocystus mimicus	Eusocial	Microsatellite analysis	0.053	Eriksson et al., 2019
Formica podzolica	Eusocial	Microsatellite analysis	0.156	DeHeer and Herbers, 2004
Oecophylla smaragdina	Eusocial	Microsatellite analysis	0.08	Schlüns et al., 2009
Ponerinae				
Neoponera inversa	Eusocial	Microsatellite analysis	−0.036 (2007)	Heinze et al., 2001; Kolmer et al., 2002; Kellner et al., 2007
Neoponera villosa	Eusocial	Microsatellite analysis	0.024	Kellner et al., 2007
Myrmeciinae				
Myrmicia pilosula	Eusocial	Microsatellite analysis	0.088	Qian et al., 2012
Myrmicia rubra	Eusocial	Microsatellite analysis; Isozyme analysis	0.041 (1982)	Pearson, 1982, 1983; Seppä and Walin, 1996
Dolichonderinae				
Iridomyrmex purpureus	Eusocial	mtDNA analysis	Not reported	Carew et al., 1997

Where available, we report r-values for comparisons among adult female nestmates, often foundresses.

can form when a pleometrotic queen association extends past worker emergence and into colony maturity. This results in primary polygyny, a group of unrelated worker lineages that share a nest, colony resources, and colony tasks. Importantly, workers in polygynous colonies may be close kin if they were produced by the same queen. Nevertheless, overall worker nestmate relatedness is often low in polygynous colonies (DeHeer and Herbers, 2004; Kellner et al., 2007). More importantly, the queens themselves represent prominent examples of non-kin cooperative behavior, analogous to cooperative breeders in other taxa, regardless of offspring group relatedness. Primary polygyny is generally found interspersed between monogynous colonies or as the majority structure in discrete populations, but has never been documented as the only social structure of an ant species.

Primary polygyny is represented in several ant subfamilies but is especially well documented in the Myrmicinae. Moser and Lewis (1981) first observed multiple unrelated queens in mature colonies of the Texas leaf-cutter ant *Atta texana*. Mintzer and Vinson subsequently found that these cooperative associations are stable and beneficial to *A. texana* queen survival in the lab (Mintzer and Vinson, 1985; Mintzer, 1987). Shortly afterward, Rissing et al. (1989) utilized allozyme markers to directly show that cohabiting *Acromyrmex versicolor* queens were not related and also reared stable multi-queen colonies in the lab. There is also genetic evidence, using isoenzymes, that two South American Acromyrmex species practice primary polygyny, *A. striatus* and *A. heyeri* (Diehl et al., 2001). Multiple, unrelated queens were also found in colonies of *Myrmica gallienii* using enzyme electrophoresis (Seppä, 1996), however colony age was not reported in this study. Primary polygyny may also occur in the fungus growing ant species, *Cyphomyrmex transversus*. Multiple queens were found in 37.7% of colonies examined by Ramos-Lacau et al. (2012) but it is unknown if these queens were related. Within the Myrmicinae, there are also several harvester ant species that practice primary polygyny. *Pogonomyrmex californicus* displays primary polygyny in southern California, as confirmed with field observation (Johnson, 2004), laboratory colonies (Clark and Fewell, 2014; Overson et al., 2014), and microsatellite analysis (Overson et al., 2016). Primary polygyny also occurs in a California population of the seed harvester *Veromessor pergandei*, also confirmed using microsatellites (Helms and Helms Cahan, 2012). Queens of another species in the same genus, *Messor barbarous*, can be induced into stable cooperative associations in the lab, but no polygynous colonies have been found in the field (Provost and Cerdan, 1990).

Within the subfamily Formicinae, the honeypot ant *Myrmecosystus mimicus* also practices primary polygyny in an Arizona population as confirmed by microsatellite analysis by Hölldobler et al. (2011). The mound building ant *Formica podzolica* exhibits primary polygyny in Colorado, as suggested by field excavation (Deslippe and Savolainen, 1995) and confirmed through microsatellite analysis (DeHeer and Herbers, 2004). Finally, multiple unrelated queens have been found in mature colonies of the pleometrotic weaver ant *Oecophylla smaragdina*, strongly suggesting primary polygyny (Schlüns et al., 2009).

Some of the most detailed genetic and behavioral research has been performed on species in the Ponerinae subfamily. Primary polygyny has been confirmed in *Neoponera inversa* through behavioral observation in the field and lab (D'Ettorre et al., 2005) as well as with multiple microsatellite analyses (Heinze et al., 2001; Kolmer et al., 2002). In a closely related species, *Neoponera villosa*, queen cooperation has been demonstrated in the lab (Trunzer et al., 1998) and unrelated queens have been documented in field colonies (Kellner et al., 2007), strongly suggesting primary polygyny. Mature *Neoponera striata* Smith colonies have also been found with multiple queens, but more work is needed on queen relatedness to confirm primary polygyny (Rodrigues et al., 2011). The arboreal trap jaw ant *Odontomachus hastatus* has been found in colonies containing several queens and workers, but it is unknown if these queens are related (Oliveira et al., 2011).

Primary polygyny has also been confirmed via microsatellite analysis in two species of the Myrmeciinae: the Australian jumper ant *Myrmicia pilosula* (Qian et al., 2012) and the red ant *Myrmicia rubra* (Pearson, 1982, 1983; Seppä and Walin, 1996).

Finally, in the Dolichoderinae subfamily, Hölldobler and Carlin (1985) found that the Australian meat ant *Iridomyrmex purpureus* is oligogynous, i.e., multiple queens share a nest but do not tolerate each other and relegate themselves to different areas of the nest. Further genetic analysis confirmed that oligogynous *I. purpureus* queens are unrelated and share a workforce (Carew et al., 1997). Oligogyny has also been documented in the subfamily Formicidae (*Camponotus ligniperdus,* Gadau et al., 1998; *Camponotus herculeanus*, Seppä and Gertsch, 1996).

ECOLOGICAL DRIVERS OF NON-KIN SOCIALITY

Group living may have its evolutionary origins across a particular set of ecological conditions that favor nest sharing and/or cooperation (Arnold and Owens, 1997; Krause and Ruxton, 2002; Rubenstein and Abbot, 2017). For non-kin groups especially, local ecology may be a prominent driver of group formation in the absence of strong indirect fitness benefits. Below, we discuss evidence for the evolution of non-kin sociality in the Hymenoptera as driven by five major ecological conditions/constraints: (1) predator and parasite pressures, (2) intraspecific competition, (3) physiological constraints, (4) productivity constraints, and (5) climatic stressors. Importantly, the distinctions we make between these five factors do not represent mutually exclusive conditions; rather, they are highly interactive and may even represent flip sides of the same environmental selective pressures (e.g., productivity constraints that arise from intense intraspecific competition). Together, these conditions may give rise to fitness differentials between solitary and social individuals when benefits of group living outweigh intrinsic costs of resource sharing.

Predator and Parasite Pressures

The need for communal defense represents one prominent benefit of nesting with non-kin. In particular, social defensive strategies often arise in contexts where brood is vulnerable to predation or parasitism (Alexander, 1974; Krause and Ruxton, 2002; Ward and Webster, 2016). Importantly, social nest defense can be a passive, emergent property of shared nesting rather than actively cooperative behavior. The presence of multiple females (or even males; Kukuk and Schwarz, 1988) in the nest can deter invaders by decreasing the daily time window in which the nest is unattended (Lin and Michener, 1972; Wcislo and Tierney, 2009). In other cases, labor may be divided such that guarding is a functional role of certain group members, often subordinates (Hogendoorn and Velthuis, 1995; Dunn and Richards, 2003). Indeed, task specialization on guarding can even emerge spontaneously among forced, unrelated associations of normally solitary individuals, suggesting that improved nest defense can arise in in communal nests from existing behavioral repertoires (Jeanson et al., 2005; Holbrook et al., 2009, 2013).

Although predator/parasite pressures have been broadly implicated in social evolutionary transitions (Michener and Lange, 1958; Lin and Michener, 1972; Krause and Ruxton, 2002; Wilson and Hölldobler, 2005), empirical demonstrations of the effectiveness of group defense in non-kin systems are sparse. For the sweat bee *Agapostemon virescens*, Abrams and Eickwort (1981) found that communal nests were more effectively defended against the cleptoparasite *Nomada articulata* than were solitary nests. Indeed, Lin and Michener (1972) consider parasite/predator pressures to be the major driver of sociality in the Halictidae (see also Michener and Lange, 1958). Similarly, co-founding wasps may experience reduced predation from birds and mammals relative to solitary foundresses, likely due to more continuous nest guarding (Strassman et al., 1988; Tindo et al., 2008). For other non-kin groups, guarding may function to repel conspecific intruders, but may not be an effective defense against predation and parasitism. For the facultatively social bees *Xylocopa virginica* and *Halictus ligatus*, rates of brood parasitism by Bombyliid flies were found to be no different between solitary and social nests, despite increased guard presence in social nests (Richards and Packer, 1998; Prager, 2014). Similarly, though multiple *Polistes* wasp foundresses may provide effective protection against intraspecific usurpation (Gamboa, 1978; Gamboa et al., 1978; Klahn, 1988), they may be no more effective in guarding against predators (Gamboa, 1978; Gamboa et al., 1978; Gibo, 1978) and parasites (Gamboa et al., 1978) than solitary foundresses, despite more continuous guard presence (Gamboa et al., 1978). However, co-founding may provide important benefits during recovery from predation attempts (Gibo, 1978; Strassman et al., 1988).

Intraspecific Competition and Resource Limitation

Grouping may arise as a response to limiting resources, especially nesting sites and food (Emlen, 1982; Hatchwell and Komdeur, 2000). Environments characterized by strong intraspecific competition may favor cooperative strategies that allow groups to exploit resources. In many cases grouping occurs in densely populated or saturated environments. Indeed, pleometrosis and primary polygyny in ants have been associated in several species with high population density (Tschinkel and Howard, 1983; Rissing and Pollock, 1986, 1991; Bennet, 1987; Trunzer et al., 1998). Likewise, for the facultatively polygynous harvester ant, *Pogonomyrmex californicus*, sites dominated by polygyny have higher colony density than primarily monogynous sites (Haney and Fewell, 2018). Further, colonies in the polygynous population have lower reproductive output than colonies from the monogynous population. Experimental food supplementation increased reproductive output of polygynous colonies to that of colonies from the monogynous population, suggesting that competitive, food-scarce conditions drive cooperation in this species (Haney and Fewell, 2018). Similarly, bees may adopt non-kin social strategies under food-scarce conditions, even in the absence of productivity benefits of group living. For the facultatively social carpenter bee *X. pubescens*, solitary nests typically outperform social nests in terms of reproductive output, due to brood mortality that results from dominance competitions in social nests (Hogendoorn, 1991, 1996). However, under conditions of food scarcity, social nesting can provide an important safeguard against pollen robbery, outweighing costs of nest sharing (Hogendoorn, 1991; Hogendoorn and Velthuis, 1993).

Nest sites can also be major limiting resources, favoring social strategies that enable nest sharing and/or increase the likelihood of nest inheritance. Carpenter bees are strongly limited by access to nest sites, creating intense competition for constructed nests that results in frequent supersedure and usurpation (Gerling et al., 1989; Buchmann and Minckley, 2019). Social nesting could feasibly provide an important defense against the threat of nest invasion, but empirical studies have demonstrated that guards of *X. pubescens*, though potentially valuable in preventing pollen robbery, do not effectively defend the nest from usurpers (Hogendoorn and Velthuis, 1993, 1995). Instead, subordinate joiners are likely hopeful reproductives that queue for reproductive opportunities upon the death of the dominant bee and subsequent nest inheritance (Hogendoorn and Velthuis, 1995; Richards and Course, 2015; Vickruck and Richards, 2018). Nest inheritance is likewise important for co-founding wasps (Reeve, 1991; Leadbeater et al., 2011), especially for species that reuse old nests (Queller and Strassmann, 1988). Similarly, for many communal bees, group living enables shared exploitation of valuable nest sites (Michener, 1974). In all these cases, intraspecific competition for nests promotes group living and interacts with other ecological constraints, especially energetic and labor constraints on nest construction.

Energetic and Physiological Constraints

Non-kin groups may also form in contexts that impose steep physiological costs on independent breeders. For example, animals that invest in energetically costly nest building behaviors may experience selection for strategies that reduce founding costs, such as cooperative building and/or nest inheritance (Hansell, 1987). Cooperative nest building has been documented broadly across Hymenopteran non-kin groups (West-Eberhard, 1969; Bartz and Hölldobler, 1982; Tschinkel and Howard, 1983; Rissing and Pollock, 1986; Peeters and Andersen, 1989; Danforth, 1991; Bernasconi and Strassmann, 1999; Hunt and Toth, 2017). In some cases, these benefits have been linked to ecological conditions and energetic constraints. The ground-nesting communal bee *Perdita portalis* excavates nests through a dense, clay layer of soil, prompting Danforth (1991) to propose energetic costs of nest construction as a major driver of sociality in this environment. Challenging excavation through hard soil may likewise favor cooperative nest construction strategies in the communal bee *Macrotera texana* (Danforth et al., 1996). Carpenter bees may also face particularly high energetic costs of nest building, due to the tendency of many *Xylocopa* species to nest in dense wood substrate. For the carpenter bee *X. sonorina*, the energetic cost of new nest construction is higher on average than the cost of nest inheritance, even accounting for the potential cost of renovating overused tunnels (Ostwald et al., 2021b). In this group, and more broadly, high costs of nest building can underlie intraspecific competition for existing nests. These costs may incentivize social strategies such as reproductive queuing or communal nesting, even at the expense of uncertain reproductive opportunities.

Beyond energetic costs, nest building behavior can impose physiological wear and damage. In arid habitats, nest construction behaviors could be constrained more by desiccation risk than by energetic costs. For many desert ants, nest excavation causes cuticular abrasion that increases water loss rates (Johnson, 2000), exacerbating desiccation risk, which is a major cause of foundress mortality (Johnson, 1998). Cooperative nest excavation during founding poses an important possible solution to this challenge. However, the physiological costs of excavation may not be shared equally among co-foundresses (Fewell and Page, 1999). Cahan and Fewell (2004) measured excavation task specialization in experimental pairs of the facultatively polygynous *Pogonomyrmex californicus*, with foundresses collected either from a typically group-founding or typically solitary-founding population. For both populations, more than half of foundress pairs divided excavation labor asymmetrically, with one foundress emerging as an excavation specialist. However, pairs from the group-founding population showed smaller asymmetries in excavation performance (Cahan and Fewell, 2004). These findings suggest that while some foundresses may experience disproportionate costs of excavation, cooperative strategies overall can reduce physiological costs of excavation for a significant portion of the population. Cooperative nest excavation and maintenance may likewise be important for some ground-nesting social bees (Danforth, 1991), but the extent to which nest excavation behavior is physiologically constrained in these groups is still unclear.

Productivity Constraints

Cooperation among non-kin can also improve productivity under harsh or competitive conditions. In particular, cooperative founding may provide competitive advantages in conditions that favor rapid nest establishment via worker production. Group founding in ants has been associated both with faster initial worker production and accelerated colony growth (Tschinkel and Howard, 1983; Rissing and Pollock, 1987; Deslippe and Savolainen, 1995; Eriksson et al., 2019; Ostwald et al., 2021c). Rapid production of a large workforce may beneficially accelerate incipient groups through the vulnerable founding period, providing a critical survival advantage for cooperatively founded colonies (Clark and Fewell, 2013; Ostwald et al., 2021c). These advantages may be especially important for colonies vulnerable to intraspecific brood raiding. Cooperative founding has been shown to improve colony survival and success during brood raiding, likely due to the protective effect of larger colony sizes (Bartz and Hölldobler, 1982; Rissing and Pollock, 1987, 1991; Eriksson et al., 2019). Increased colony size in multi-foundress nests is also associated with reduced colony failure rates for the paper wasp *Polistes dominula* (Tibbetts and Reeve, 2003). Importantly, cooperative foundresses may experience enhanced colony growth without increasing costly individual investment in sterile worker production. Multi-queen colonies of the harvester ant *P. californicus* experience faster colony growth than single queen colonies, but lower per-queen worker production (Ostwald et al., 2021c). The ability to assemble a large workforce while minimizing individual investment in non-reproductive offspring may represent an important physiological benefit of cooperation with non-relatives.

Specifically, individuals may face productivity constraints associated with resource exploitation. For example, the communal bee *Macrotera texana* faces severe reproductive time constraints due to its foraging dependence on *Opuntia* flowers that bloom for only 2–3 weeks per year (Danforth et al., 1996). Cooperative nest excavation likely enables females to exploit this time-limited resource by accelerating nest founding (Danforth et al., 1996). Similarly, increased colony activity levels in polygynous *P. californicus* colonies suggests both increased worker production and corresponding enhanced efforts to capitalize upon limiting food resources (Haney and Fewell, 2018). In this way, productivity constraints interact strongly with resource limitation and intraspecific competition.

Importantly, worker production benefits may not translate to enhanced production of reproductives. For *P. californicus* as well as for the sweat bee, *Halictus ligatus*, group-founding nests produce more workers but fewer reproductive offspring than solitary-foundress nests (Richards and Packer, 1998; Haney and Fewell, 2018). *Polistes* foundress associations are likewise associated with reduced per-capita reproductive output (Queller and Strassmann, 1988; Reeve, 1991), despite increased worker production in some species (Tibbetts and Reeve, 2003). These cases suggest that cooperation often functions not as a means to enhance reproductive output under ideal conditions, but rather as a strategy to minimize losses under constraining or challenging environmental conditions.

Climatic Stressors

Climatic factors represent fundamental ecological drivers of group living across animal taxa. In particular, cooperation may be favored in harsh or stochastic climates (Arnold and Owens, 1997; Jetz and Rubenstein, 2011; Rubenstein, 2011; Griesser et al., 2017; Lukas and Clutton-Brock, 2017; Kennedy et al., 2018). In insects, climate likewise mediates the expression of social behavior, especially through impacts on development time and seasonal activity windows, which affect the available time for rearing workers and therefore the potential for colony life to emerge (Eickwort et al., 1996; Hunt and Amdam, 2005; Hirata and Higashi, 2008; Fucini et al., 2009). These factors may be important in the evolution of eusociality by promoting generation overlap in the nest. For non-kin groups, however, that arise from stable cooperative relationships between unrelated individuals, the effects of climate on group formation are relatively unexplored.

Nevertheless, several studies point to prominent roles for climatic conditions, especially environmental temperatures, in facilitating non-kin cooperation. Among *Polistes* paper wasps, which can found nests with non-relatives, cooperative nest founding is associated with high temperature variability, perhaps due to buffering cooperation of sociality in unpredictable environments (Sheehan et al., 2015). Polygyny in ants has also been associated with harsh thermal environments (Heinze, 1993; Heinze and Hölldobler, 1994; Heinze and Rüppel, 2014) and with success of invasive species in their introduced environments (Holway et al., 2002; Tsutsui and Suarez, 2003). Future work should clarify mechanisms underlying this link between cooperation and success in harsh, variable, or novel thermal environments.

Precipitation can also influence the relative costs and benefits of grouping. Arid environments and drought conditions can increase soil hardness, potentially increasing excavation costs and exacerbating nest limitation for ground nesting bees, ants, and wasps (Wcislo, 1997; Michener, 2007; Purcell, 2011). Under drought conditions, Bohart and Youssef (1976) found that 30% of nests of the normally solitary sweat bee *Lasioglossum galpinsiae* were provisioned by multiple females. In desert ants, group founding may be a by-product of the tendency to seek refuge from desiccating conditions in shared belowground spaces (Pfennig, 1995). Under desiccating conditions, group-founding by the desert seed-harvester ant *Veromessor pergandei* enhanced queen survival and water content relative to solitary queens, though the mechanism for this advantage is unclear (Johnson, 2021). Shared foraging duties could feasibly reduce risk of desiccation in desert habitats. Cahan and Fewell (2004) suggest that a group-founding population of the harvester ant *P. californicus* occupies a habitat with lower and less predictable summer precipitation than sites occupied by solitary founding populations, suggesting possible desiccation constraints. In less arid habitats, extended periods of rain can cause nest failure for ground-nesting species. For the sweat bee *Halictus ligatus*, foundress cooperation may provide protection against rain-induced nest failure through enhanced nest maintenance (Richards and Packer, 1998). As such, like environmental temperature, precipitation can alternately promote or constrain cooperative behavior among non-relatives.

DISCUSSION

Sociality can be understood as an adaptive response to ecological conditions. Non-kin groups present valuable test cases for hypotheses about the ecological drivers of group formation, in particular, because communal and co-founding strategies are nearly always facultative at the individual or population level (Ross and Matthews, 1991; Michener, 2007; Heinze et al., 2017).

Studying non-kin groups usefully controls for indirect fitness benefits, thus enhancing our understanding of other, relatively neglected drivers of group formation. These systems have yielded important intraspecific demonstrations of the role of ecology in determining the adaptive value of grouping behavior. Here, we have explored five central ecological factors expected to interact with the expression of social behavior: interspecific pressures from predators and parasites, intraspecific pressures over limited resources, environmental constraints on individual physiology and productivity, and stressors associated with climate. Evidence from across Hymenopteran systems indicates that these conditions play a pivotal role in shaping non-kin social strategies.

Importantly, these ecological drivers of sociality are highly interactive. Efforts to understand sociality across a single environmental axis are limiting and often yield contradictory results (e.g., sociality alternately increasing and decreasing with latitude; Purcell, 2011). Instead, integrative approaches that accommodate these interactions can provide important insights into the complex conditions underlying grouping responses. Studies in Hymenopteran systems have emphasized interactions among intraspecific, interspecific, and abiotic selective pressures. For example, sociality can be a response to intraspecific competition for access to nests (Gerling et al., 1989; Leadbeater et al., 2011). This competition is often a direct product of physiological constraints associated with nest construction behavior (Johnson, 2000; Ostwald et al., 2021b), which can be exacerbated by climatic stressors such as low precipitation (Wcislo, 1997; Purcell, 2011). This particular nexus of challenges is an important driver of group formation among the communal and parasocial bees and polygynous ants (Danforth, 1991; Danforth et al., 1996; Cahan and Fewell, 2004). Highly competitive environments can also give rise to cooperative strategies that mitigate worker production constraints experienced by solitary foundresses. Accelerated worker production is a major benefit of cooperation among ant foundresses vulnerable to brood raiding in contexts dominated by intraspecific competition (Bartz and Hölldobler, 1982; Rissing and Pollock, 1987, 1991; Eriksson et al., 2019). Productivity constraints may also be important drivers of grouping in environments dominated by predation pressures; for group-founding wasps, increased colony sizes can provide essential resilience following predation attempts (Strassman et al., 1988). Together, these examples suggest shared sets of ecological conditions that favor cooperative behavior even when relatedness is low or absent among group members. Importantly, these conditions are not restricted geographically but instead occur at intersections of particular selective pressures that can occur across a wide variety of habitat types.

These findings in non-kin groups of ants, bees, and wasps parallel known drivers of social evolution in non-insect social systems, both kin and non-kin. Ecological constraints are prominent, known drivers of cooperative breeding in birds and mammals (Emlen, 1982, 1984; Arnold and Owens, 1997; Hatchwell and Komdeur, 2000; Shen et al., 2017). Inheritance tactics in nest-limiting environments may favor delayed dispersal and nest joining (Woolfenden and Fitzpatrick, 1978; Emlen, 1984). As with the ground-nesting ants and bees, nesting constraints may be physiological, and can be exacerbated by climatic conditions: nest excavation costs in arid conditions have been proposed as a major driver of sociality in the African mole-rats (Jarvis et al., 1994; Faulkes et al., 1997; Hansell, 2005). More broadly, low and unpredictable rainfall has been associated with the global biogeography of cooperatively breeding mammals (Lukas and Clutton-Brock, 2017). Environmental stochasticity has also been implicated in the global distribution of cooperative breeding in birds (Jetz and Rubenstein, 2011), suggesting important links between cooperation and environmental uncertainty that parallel trends described in *Polistes* foundress associations (Sheehan et al., 2015).

Strengthening the conceptual links among Hymenopteran and vertebrate sociality has great potential for the development of broader evolutionary frameworks explaining non-kin cooperation. Vertebrate research has benefited from a more comprehensive understanding of the taxonomic distribution of kin and non-kin sociality, especially among the cooperatively breeding birds. This knowledge base has enabled valuable phylogenetic studies highlighting the roles of environmental and life history factors in shaping social organization (Riehl, 2013; Downing et al., 2015, 2020; Cornwallis et al., 2017). The social Hymenoptera likewise present special opportunities to study non-kin sociality because it occurs frequently across closely related lineages. To our knowledge, this comparative approach has not yet been applied to the Hymenoptera in the context of kin vs. non-kin social evolution, but may be feasible for those taxa in which non-kin sociality is better documented, especially the polygynous ants.

Beyond this comparative framework, the literature on vertebrate social systems can provide social insect researchers with valuable approaches for studying direct benefits of cooperation. The social vertebrate literature is rich in explorations of the costs and benefits of well-defined cooperative behaviors, from hunting and defending food (Packer and Ruttan, 1988; Lucas and Brodeur, 2001) to detecting and repelling predators (Hamilton, 1971; Foster and Treherne, 1981) or successfully rearing offspring (Ebensperger et al., 2007; Hodge et al., 2009). Likewise, studies should investigate direct benefits of cooperative behaviors in Hymenopteran societies, for example, the effectiveness of nest defense in social vs. solitary bee nests (as in Hogendoorn and Velthuis, 1993; Prager, 2014), or the consequences of shared foraging duties in ant and wasp foundress associations (Cahan and Fewell, 2004). Importantly, the exchange of theories and ideas between vertebrate and invertebrate sociality research should be bi-directional. Insights from Hymenopteran systems have the potential to overcome many of the limitations of work with vertebrate systems. Especially given their short generation times and experimental tractability in lab settings, insect systems have the potential to fill gaps in our broader understanding of the long-term direct fitness outcomes of cooperation over multiple generations.

Current understanding of social evolution among unrelated individuals is constrained by limited knowledge of the full diversity of Hymenopteran taxa that form non-kin groups. The incidence of non-kin cooperation is likely to be greatly underestimated due to the tendency of non-kin groups to occur within otherwise solitary populations (Ross and Matthews, 1991; Michener, 2007; Heinze et al., 2017), and due to limitations associated with quantifying relatedness in some species. This knowledge gap can be addressed with simple behavioral techniques (e.g., mark-recapture or observations of nest joining; Abrams and Eickwort, 1981; Peso and Richards, 2011) and inexpensive genotyping methods (e.g., microsatellites; Moore and Kukuk, 2002). Other techniques, like radio-frequency tracking (Sumner et al., 2007; Kissling et al., 2014), have the potential to reveal nest switching patterns that maintain low relatedness in some insect groups. A first priority in future research on non-kin sociality should be to expand our understanding of the diversity of non-kin systems via integrated behavioral and molecular research. Many of the species highlighted in **Table 1** currently possess incomplete evidence for non-kin sociality, especially among the wasps and bees. It is likely that non-kin groups form among many other, related species for which kinship has not yet been quantified. The same may be true for similarly structured social groups outside the Hymenoptera, especially among the termites, which can form polygynous colonies through colony fusion (DeHeer and Vargo, 2004, Deheer and Vargo, 2008; Korb and Roux, 2012).

Beyond characterizing the organization and formation of these groups, studies that relate social founding strategies to ecological conditions or compare social and solitary strategies in sympatry represent promising directions for future research. Particularly illuminating would be controlled experimental studies relating social condition to ecological conditions and, especially, to fitness outcomes. The abundance of facultatively social non-kin groups provides diverse, experimentally tractable systems in which social condition can be observed and even manipulated within a single species, thus avoiding the pitfalls of comparisons across species with very different evolutionary histories. Manipulative studies such as these could rigorously test hypotheses about proposed drivers of sociality, providing insights into the ecological conditions at the origins of group living.

CONCLUSION

The ecological drivers of non-kin cooperation represent a highly overlapping suite of conditions that interact to constrain solitary reproductive opportunities. Integrative research that accommodates these interactions has the potential to reveal common principles underlying social evolution broadly across animal taxa and across kin and non-kin groups. Our current understanding of the full diversity of non-kin sociality in the Hymenoptera is highly limited, but existing analyses suggest that groups containing non-relatives are more widespread than previously acknowledged. Future work should quantify relatedness across a diversity of species, and leverage these systems as models for evaluating the ecological conditions that favor group formation. Studies of known non-kin groups in the Hymenoptera have emphasized the role of harsh, competitive environments in selecting for cooperative strategies even in the absence of indirect fitness benefits. These findings parallel patterns more broadly across animal groups that indicate a major role for ecological constraints in shaping diverse forms of sociality.

AUTHOR CONTRIBUTIONS

MO wrote the main manuscript. BH wrote the section on ant sociality. All authors contributed to manuscript editing and developing the concept for the review.

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Efficient Allocation of Labor Maximizes Brood Development and Explains Why Intermediate-Sized Groups Perform Best During Colony-Founding in the Ant, *Pogonomyrmex californicus*

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Cooperation in nature is usually between relatives, but unrelated individuals can also cooperate, requiring significant benefits to outweigh the costs of helping non-kin. Unrelated queens of the ant, *Pogonomyrmex californicus*, work together to found a new colony, a phenomenon known as pleometrosis. While previous studies have shown that pleometrosis improves queen survival and worker production, little is known of the behavioral interactions within nests that explain these advantages. We aimed to determine how the optimal group size for a small, simple social group is related to group productivity and the organization of work. Collecting queens from a known pleometrotic population, we established nests with either one, three, six, or nine foundresses and observed the resulting nascent colonies for 50 days. We found that queens in social founding groups survived longer and had higher productivity. While all social groups were equally successful in producing workers, intermediate-sized groups were most successful in terms of per capita production. Inactivity increased with group size. In addition, the proportion of essential colony growth tasks performed (such as foraging and brood care) was lowest in both solitary-founded groups and in groups of nine queens. As a result, intermediate sized groups outperformed both solitary queens and groups of nine in the efficiency with which they converted eggs into workers. These results emphasize the benefits of cooperation and the ways in which group size can influence fitness and the allocation of labor in social groups.

Keywords: ant foundresses, cooperative nest-founding, pleometrosis, *Pogonomyrmex californicus*, seed-harvester ants

INTRODUCTION

A central question in social biology is that of why groups form. Group membership should ultimately enhance an individual's inclusive fitness. However, cooperation introduces costs. By contributing to the group, individual members may sacrifice some "market share" of direct fitness. In many contexts, these costs can be offset by indirect gains, particularly in groups with significant relatedness. The question of group formation becomes particularly interesting, however, when cooperators are not relatives (Dugatkin, 2002; Clutton-Brock, 2009; Taborsky et al., 2016). In such cases, indirect fitness moves toward zero, and direct fitness becomes the primary focus of selection.

The fitness costs and benefits of cooperation are also tied to group size (Dornhaus et al., 2012). Animal societies range widely in size, with important effects on survival and reproduction. For example, social grouping can influence thermoregulation (Cook and Breed, 2013). For animals that hunt in groups, optimum group size must balance the benefit of collective prey capture with the cost of more mouths to feed (Caraco and Wolf, 1975; Yip et al., 2008). Resource defense and interspecific competition can also be important factors shaping the optimum group size (Adams and Tschinkel, 1995). Group size can also limit the benefits of cooperation, for example in increased vulnerability to predators or pathogens (Côté and Poulinb, 1995). Thus, natural selection may ultimately act to "tune" social organisms toward an optimum group size and associated fitness-maximizing behaviors (Avilés and Tufino, 1998; Yip et al., 2008).

Cooperative nest founding among unrelated ant queens is a useful context to study the interplay between group size and individual fitness, because queens of some populations form small cooperative groups that are readily studied (Hölldobler and Wilson, 1977; Strassmann, 1989). In many species of social insect, independently founding reproductives can form cooperative associations. Known as pleometrosis, this phenomenon has been documented in ants (Sommer and Hoelldobler, 1995; Choe and Crespi, 1997; Bernasconi and Strassmann, 1999; Johnson, 2004; Izzo et al., 2009; Offenberg et al., 2012; Eriksson et al., 2019), bees (Schwarz et al., 1998), wasps (Rau, 1931; Itô, 1986), termites (Hacker et al., 2005; Chiu et al., 2017), mites (Saito, 2009), aphids (MillerIII, 1998a; Miller, 1998b; Michaud and Belliure, 2000), and thrips (Crespi et al., 1997; Gilbert and Simpson, 2013). When cooperation persists throughout the life of the colony, it is called primary polygyny—as in the case described in this paper (Ostwald et al., 2021).

A number of benefits from pleometrosis have been found in ants, including: (1) decreased foundress mortality (Waloff, 1957; Tibbetts, 2003; Johnson, 2004), (2) earlier production of workers, (3) larger nascent colony size, which may be of benefit in interspecific competition or against predators (Gamboa, 1978; Bartz and Hölldobler, 1982; Tschinkel and Howard, 1983; Thorne, 1984; Rissing and Pollock, 1987; Adams and Tschinkel, 1995; Jerome et al., 1998; Tierney et al., 2000), (4) protection from predators and parasites during brood development (Abrams and Eickwort, 1981; Soucy and Giray, 2003), (5) and in intraspecific competition (Izzo et al., 2009). However, cooperative colony foundation can be costly for the same reasons as outlined for other social groups, including loss of reproductive opportunities and pathogen and parasite transmission risks (Cahan and Julian, 1999). In spite of these challenges, pleometrosis has evolved frequently in ants and other taxa.

In several species of pleometrotic ants, the success is not a simple multiple of the number of queens, but instead peaks at foundress numbers between 4 and 7 (Bartz and Hölldobler, 1982; Tschinkel and Howard, 1983). While these studies showed that mid-sized foundress groups have a survival and production advantage, the exact behavioral causes of this phenomenon remain to be investigated.

The seed harvester ant, *Pogonomyrmex californicus*, is haplometrotic (single queen founding) through much of its range. However, several populations of pleometrotic colony foundation have been identified (Johnson, 2004). Although *P. californicus* has become a model system for questions surrounding the evolution of cooperation and division of labor (Cahan and Julian, 1999; Jeanson and Fewell, 2008; Waters et al., 2010; Dolezal et al., 2012; Clark and Fewell, 2014), the majority of studies of *P. californicus* have focused on foundress pairs. Field studies indicate that the number of nest foundresses and stable matrilines in mature colonies tends to an average of four per nest (Overson et al., 2014, 2016). In spite of the artificial conditions of these previous studies (which utilized ant farms and/or buckets of dirt as a nesting substrate), fitness effects of different group sizes and pairing combinations have often been demonstrated. For colonies in a natural setting, most of the life of the queens takes place in the confines of small nests (besides foraging)—making the ant farm method a reasonable proxy for early colony life.

Using ants from the same population described by Overson et al. (2014, 2016), we created groups of foundresses ranging in size from 1 to 9 queens and observed them for 50 days in ant farms. Our study posed a set of three related questions: (i) How does group size influence foundress survival? (ii) Does foundress number influence nest productivity? (iii) How does foundress number influence individual activity levels and task performance? By answering these questions, we explore the behavioral factors contributing to group success or failure across group sizes below and above the mean field number. We also examine the regulatory mechanisms, in terms of behavior and allocation of labor, that underlie the success of pleometrotic foundress groups.

MATERIALS AND METHODS

We collected newly mated *Pogonomyrmex californicus* foundresses from the Pine Valley area in San Diego, California (32.821 N, 116.529 W) between July 2 and 4, 2009. This population is known to be pleometrotic (Rissing et al., 2000; Cahan and Fewell, 2004; Johnson, 2004; Overson et al., 2016). Queens were collected from the ground after they had removed their wings but before they began to excavate nests.

In the lab, we weighed individual foundresses and assigned each one to a group of one, three, six or nine queens by haphazard selection. After group assignment, each queen was paint-marked on the abdomen for individual identification and then placed into observation nests. We created 30 nests per treatment for a total of 120 nests and 570 foundresses.

Observation nests were composed of two 15 × 20 cm glass plates (2 mm thick) separated by 3.5 mm thick plastic siding on all four sides. The top piece of siding acted as a lid to slow down soil drying and prevent ant escapes. We filled nests with sifted soil from the foundress retrieval site. Nests were soaked before use by submersing the bottom in water until the soil was evenly moist.

Initial moisture levels were high enough that regular watering was not necessary; those nests that became dry over the course of the experiment were provided with additional moisture at day 35 by watering above the nest surface. Food for each nest was provided (approximately 3 Kentucky bluegrass seeds per foundress) every 3 days. The laboratory was maintained at a constant 30°C throughout the experiment, with natural summer day/night light cycles. The foundresses excavated tunnels that spanned the entire width of the ant farm, such that ants and brood were always visible. Brood counts were performed by temporarily laying the ant farm flat on a table and observing with a binocular microscope, with bright light provided by a 150-Watt halogen fiber optic light source.

We began behavioral observations at 8:00 a.m. on July 6, the day following nest creation. Over Days 1–4, nests were observed approximately five times per day with scan sampling to note task performance and locations for each foundress. From Days 5 to 50, the behavior of each queen was monitored and recorded once per day. To monitor task performance and social interactions, we recorded each behavior performed. **Table 1** shows the full list of behaviors that were recorded, along with their definitions and classification with regards to whether or not each behavior was considered as contributing toward "colony growth work."

Nests were monitored for queen and nest survival each day throughout the experiment. A nest was considered alive if at least one living queen remained. Only two instances of aggression (biting) were documented between foundresses: one event in a group of three and another event in a group of nine. Neither event resulted in mortality. Production of eggs, larvae, and pupae was tracked for each nest every 3 days, starting at Day 3. The date of first worker eclosion for each nest was also recorded, and following worker eclosion, the number of workers also counted following the 3-day schedule. In measuring brood production, we aimed to quantify not only the overall output of brood per nest, but also brood production per starting foundress. In addition, we calculated brood conversion efficiency by dividing the number of eggs (or other brood items) at a given stage (eggs, larvae, pupae) by the subsequent number of individual items produced at later stages (larvae, pupae, new workers). Conversion efficiencies were calculated at each developmental transition (eggs to larvae, larvae to pupae, and pupae to worker) to pinpoint the precise timing of the success or failure (developmentally speaking) of nascent colonies. Finally, the overall egg-to-worker conversion efficiency was calculated.

TABLE 1 | A summary of ant queen behaviors recorded during 50 days of observation.

		Behavior	Description
Colony growth	Active	Tunneling	Located in the tunnel, and digging with the mandibles
		Excavating	Carrying soil from the tunnel to the surface
		Tending brood	Actively antennating or grooming the brood pile
		Foraging	Collecting/carrying seeds
		Undertaking	Picking up or moving a dead foundress
		Guarding	Maintaining a stationary position at the mouth of the nest
		Allogrooming	Cleaning another foundress
Non-colony growth		Biting	Aggressive interactions with other ants
		Inactive	No movement or discernable activity
		Grooming	Active grooming of themselves
	Inactive	Inactive	No movement, discernable activity, or interactions with other queens

We classified behaviors as active (any identifiable nest task or motion) or inactive (motionless and not engaged in any interaction with a nestmate) (**Table 1**). To characterize work directed toward nest construction and colony growth, we measured incidents of the following tasks: tunneling, excavating, foraging, and tending brood. Although likely relevant to colony function, walking, self-grooming, inactivity, and biting were excluded from the task analysis. We first considered the total number of growth tasks performed per nest, summed over the 50 days of observation. Then, to obtain an index of how many colony growth tasks each individual queen performed while alive, the total colony growth tasks performed by each ant was divided by the number of days the ant was present/alive ("ant days"). To get a picture of how each queen's overall activity budget depended on nest context, the proportional activity of each queen was found by dividing the total of that category by the total number of observations across the following five categories: walking, digging, foraging, brood care, and inactivity. In social groups, these proportions were averaged in each nest. Finally, each proportion was averaged across all 30 replicates to compare the typical activity budgets for groups of one, three, six, and nine foundresses.

To obtain a measure of fitness that takes into account both per capita production and survival (Shen et al., 2017), we calculated the product of per capita worker production at day 50 and the proportion of foundresses surviving to day 50.

Statistical Analysis

To assess differences in mortality we performed a Log Rank Mantel-Cox survival analysis on foundresses. The same method was used to examine nest survival. The timing of brood emergence was compared across treatments with Kruskal-Wallis tests. These analyses were performed with IBM SPSS Statistics for Macintosh, Version 25.

For comparisons of count data (numbers of brood, workers, queens, and behavioral acts), we fit generalized linear models using R version 4.0.3. To compare brood conversion rates (e.g., workers produced per egg), we used the counts of the earlier brood stage as an offset in the regression model. Similarly, to analyze per capita worker and brood production, we used foundress number as an offset. For activity rates, we used the total number of ant-days in each nest as an offset. Ant-days were calculated by summing the number of days each foundress was present in the nest (i.e., the number of days until she died or escaped). For most of these analyses, we used negative binomial regression, because Poisson regression models fit poorly due to overdispersion. *Post hoc* pairwise comparisons among groups were evaluated with Z-tests after Holm-Bonferroni correction of p-values to avoid alpha inflation.

To test for differences among foundress group sizes in the overall fitness metric (the product of survival and worker production), we fit a linear model in R. Adherence to the normality and equal variance assumptions of linear models was confirmed by inspecting residual plot and a normal probability plot of the model residuals.

RESULTS

Influence of Group Size on Foundress Survival Probability

Single foundresses experienced much higher mortality rates than did foundresses in groups of any size (Log Rank Mantel-Cox: $\chi^2 = 85.3$; df = 3; $p < 0.001$; **Figure 1**). Mean survival time for solitary foundresses was 15.8 ± 1.2 days, with none surviving past day 33. In contrast, mortality rates were similar across all social group sizes (Log Rank Mantel-Cox: $\chi^2 = 4.3$; df = 2; $p = 0.117$; **Figure 1**). Mean survival times in groups of three, six, and nine were 36.6 ± 1.9 days, 38.1 ± 1.3 days, and 39.5 ± 1.0 days, respectively.

Nest survival patterns mirrored those for individuals: solitary foundresses had significantly shorter survival times than nests founded by three, six, or nine queens (Log Rank Mantel-Cox: $\chi^2 = 133.2$; df = 3; $p < 0.001$). Social groups were similar to one another in nest survival (Log Rank Mantel-Cox: $\chi^2 = 0.6$; df = 2; $p = 0.733$).

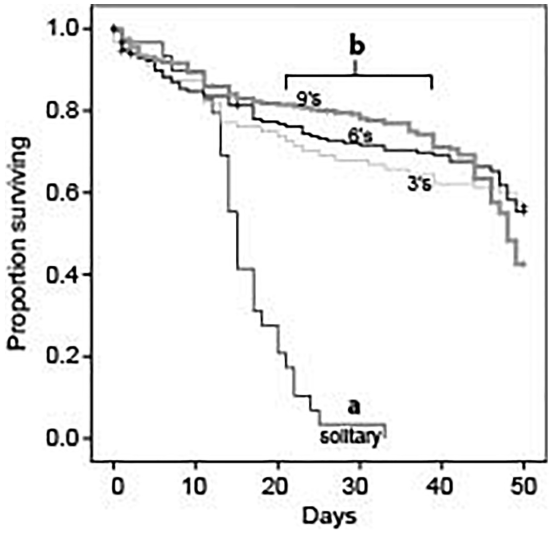

FIGURE 1 | Survival rates for queens in foundress groups established with 1, 3, 6, or 9 queens. Solitary queens had significantly lower survival rates compared to queens in groups (Log Rank Mantel-Cox, chi-square = 85.286, df = 3, $P < 0.001$). However, individual survival rates did not differ across groups that contained 3, 6, or 9 queens (Log Rank Mantel-Cox, chi-square = 4.291, df = 2, $P = 0.117$).

By the conclusion of the study at day 50, single queens had suffered 100% mortality, but most nests in social treatments still had survivors. Among the social groups, foundress number significantly affected the number of survivors (Negative binomial regression: χ^2 8.87; df = 2; $p = 0.012$). For three-queen groups, the 95% confidence interval for mean number of survivors was 1.1–2.5. For six- and nine-queen groups, the confidence intervals were 1.1–3.4 and 1.3–3.8, respectively. The mean was significantly less for groups of three than for groups of six (Z-test: $p = 0.015$) or nine (Z-test: $p = 0.003$). Groups of six and nine were similar to each other (Z-test: $p = 0.59$).

Influence of Group Size on Brood Production and Efficiency of Worker Production

Group size did not influence the timing of development; all treatments were similar in the number of days necessary for egg production and the time required to transition to larvae, pupae, and workers (**Figure 2**). Production of eggs peaked at day 9, larvae at day 19, pupae at day 34, and workers at the end of the experiment (**Figure 2**). Averages (± SE) for peak values are provided in **Table 2** and per-capita brood production at each peak are provided in **Table 3**.

Treatments differed in peak egg production at Day 9 [Negative binomial regression: $\chi^2 = 100.6$; df = 3; $p < < 0.0001$; Z-tests: $p < 0.01$ in all pairwise comparisons except for six foundresses vs. nine foundresses ($p = 0.052$)]. Larger groups produced correspondingly more eggs (**Figure 2** and **Table 2**). On a per capita basis, however, peak egg numbers per starting foundress were significantly lower for solitary foundresses compared to foundresses in groups, but similar across the three group sizes (Negative binomial regression: $\chi^2 = 18.0$; df = 3; $p < 0.001$; Z-tests: $p < 0.01$ for solitary compared with all other foundress numbers; $p > 0.5$ for all other pairwise comparisons;, **Table 3**).

Foundress number also significantly influenced the peak production of larvae at Day 19 (Negative binomial regression: $\chi^2 = 38.7$; df = 3; $p < 0.001$; **Table 2**), such that groups founded by solitary queens produced significantly fewer larvae than any of the groups (Z-test: $p < 0.001$ in all pairwise comparisons). Only three nests founded by solitary foundresses succeeded in producing any larvae at all. Among the group treatments, peak larvae numbers were similar regardless of nest size (Z-test: $p > 0.35$ in all pairwise comparisons).

In contrast to egg production, per capita larval production differed according to group size (Negative binomial regression: $\chi^2 = 28.4$; df = 3; $p < 0.001$; **Table 3**), such that three-foundress groups produced significantly more larvae per initial queen than the other three treatment groups (Z-test: $p < 0.003$ in all three pairwise comparisons). Solitary foundresses had lower per capita production than three-foundress groups (Z-test: $p = 0.02$), but were similar to groups founded by six ($p = 0.08$) or nine queens ($p = 0.17$). Six- and nine-queen groups had intermediate and similar per capita larva production (Z-test: $p = 0.052$).

Solitary queens never produced pupae or workers, so these stages were analyzed only for the social group treatments. The treatments differed significantly in peak pupae numbers at day

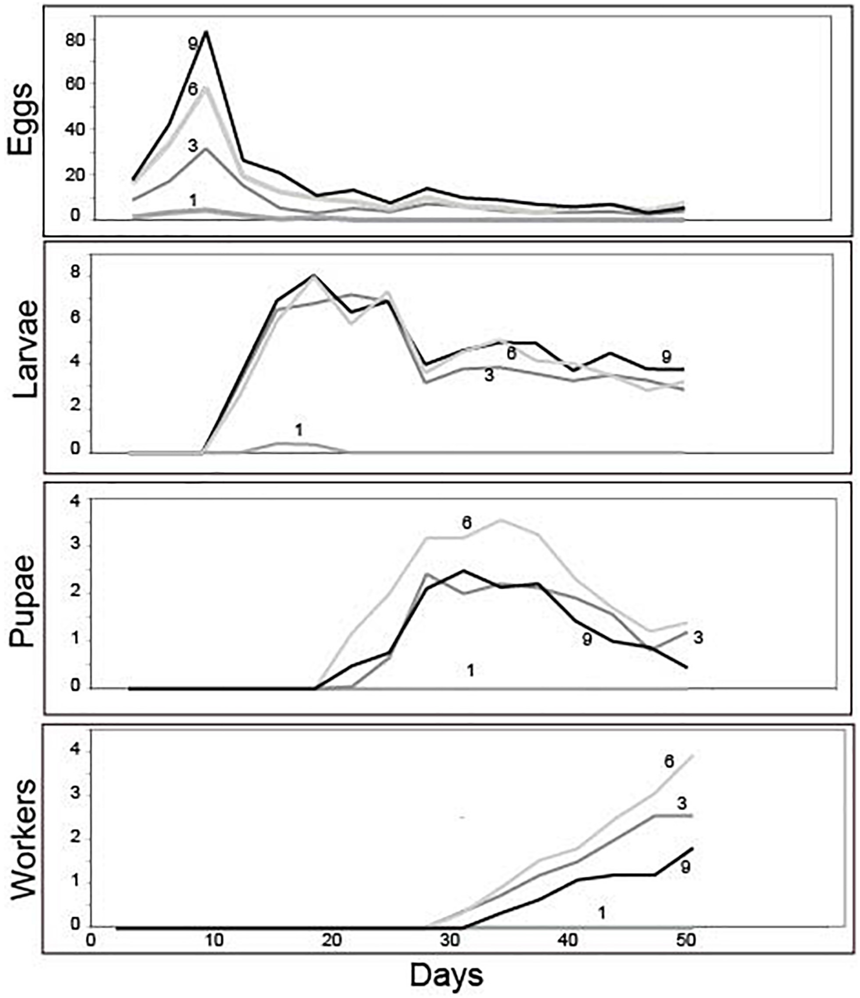

FIGURE 2 | Mean brood development trajectories over the 50-day experiment for groups founded by 1, 3, 6, or 9 queens. While the trajectory and timing of brood development was similar across groups, their success in transitioning between stages of development and ultimately producing workers depended on foundress group size (see text for statistics).

34 (Negative binomial regression: $\chi^2 = 6.6$; df = 2; $p = 0.036$). Although none of the pairwise comparisons were significantly different, six-queen groups had the highest average number of pupae (**Figure 2** and **Table 2**). On a per capita basis, groups of three and six queens showed significantly greater production of pupae than the groups of nine (Negative binomial regression: $\chi^2 = 22.2$; df = 2; $p < 0.0001$; Z-tests: $p < 0.0001$; **Table 3**), while groups of three and six were similar to each other (Z-test: $p = 0.34$).

The median date for worker emergence was similar among the social group treatments (Kruskal-Wallis: $H_2 = 2.9$; $p = 0.24$). For three-, six-, and nine-foundress treatments, workers emerged on Day 34.7 ± 5.2, 33.3 ± 3.7, and 35.8 ± 5.1, respectively ($\overline{X}\ SE$). Workers steadily accumulated in number until the conclusion of the study on Day 50, by which time workers were present in 73.3% of three-queen groups 76.6% of six-queen groups, and 60% of nine-queen groups. On Day 50, worker production clearly differed across treatments, in that no solitary foundresses produced any workers while the social groups produced one to three workers, on average. There was no significant difference among social groups in worker production (Negative binomial regression: $\chi^2 = 5.62$; df = 2; $P = 0.06$; Z-tests of pairwise

TABLE 2 | Absolute mean brood production (± SE) at peaks of development for groups founded by 1, 3, 6, and 9 queens.

Initial foundress number	Eggs (day 9)	Larvae (day 19)	Pupae (day 34)	Workers (day 50)
1	4.3 ± 1.1^{a}	0.4 ± 0.2^{a}	0 ± 0	0 ± 0
3	31.6 ± 3.1^{b}	8.0 ± 1.0^{b}	2.2 ± 0.3^{a}	2.6 ± 0.4^{a}
6	58.6 ± 6.2^{c}	6.8 ± 3.7^{b}	3.6 ± 0.5^{a}	4.0 ± 0.6^{a}
9	83.5 ± 5.4^{d}	8.0 ± 0.9^{b}	2.1 ± 0.4^{a}	1.8 ± 0.7^{a}

Statistical significance (Negative binomial regression, $p < 0.05$) is indicated by superscript letters.

TABLE 3 | Mean per capita brood production (± SE; per initial foundress) at peaks of development for groups of 1, 3, 6, and 9 queens.

Initial foundress number	Eggs/initial foundress (day 9)	Larvae/initial foundress (day 19)	Pupae/initial foundress (day 34)	Workers/initial foundress (day 50)
1	4.31 ± 1.07^{a}	0.38 ± 0.52^{a}	0.0 ± 0.0	0.0 ± 0.0
3	10.53 ± 1.03^{b}	2.25 ± 0.24^{b}	0.74 ± 0.08^{a}	0.86 ± 0.12^{a}
6	9.77 ± 1.03^{b}	1.34 ± 0.87^{a}	0.59 ± 0.08^{a}	0.66 ± 0.11^{a}
9	9.27 ± 0.60^{b}	0.89 ± 0.53^{a}	0.24 ± 0.05^{b}	0.20 ± 0.08^{b}

Statistical significance (Negative binomial regression, p < 0.05) is indicated by superscript letters.

differences: $P > 0.05$). Due to the lack of variance in one-foundress nests (all had zero workers), they could not be included in the statistical analysis. However, their difference from the social groups is clear in that none of the latter's 95% confidence intervals for mean number of workers included zero. Per capita worker production differed significantly among the social groups (Negative binomial regression: $\chi^2 = 15.7$; df = 2; $P < 0.001$) with higher levels in three- and six-queen groups than in nine-queen groups (Z-tests: $p < 0.001$). There was no significant difference between groups of three and six (Z-test; $p = 0.377$; **Table 3**).

The net worker-per-egg conversion efficiency was estimated by using counts from the respective days of peak production, and differed across the three social group treatments (Negative binomial regression: χ^2 12.5; df = 2; $p < 0.01$; **Figure 3** and **Table 4**). Groups founded by three and six queens had similar efficiencies (Z-test, $p = 0.69$), but were significantly greater than groups founded by nine queens (Z-tests, $p < 0.01$ for both comparisons). Three-queen groups produced 0.08 ± 0.06 workers per egg ($\bar{\mathbf{X}}$ **S**D) while six- and nine-queen groups produced only 0.07 ± 0.05 and 0.02 ± 0.04 workers per egg, respectively. For all three social group sizes, the 95% confidence intervals for mean worker-per-egg conversion rate excluded zero, indicating a significant advantage over one-foundress nests, none of which produced any workers.

The transition that contributed most strongly to differences in the conversion of eggs to workers was the transition from eggs to larvae (Negative binomial regression: $\chi^2 = 26.5$; df = 3; $p < 0.001$). Groups founded by three queens averaged more larvae per egg than groups founded by 1 queen or nine queens (0.2, 0.02, and 0.1, respectively; both Z-tests: $p < 0.05$). Groups of six queens produced an intermediate 0.14 larvae per egg, which was not statistically distinguishable from groups founded by one queen (Z-test, $p = 0.12$) or 3 queens ($p = 0.06$), but was significantly different from 9-queen groups (Z-test: $p = 0.05$). Finally, larvae-per-egg conversion efficiency was similar for groups founded by 1 queen compared to groups founded by 9 queens (Z-test, $p = 0.09$). At the other transitions (larvae to pupae and pupae to workers), the solitary queen group was excluded from analysis due to failure to produce pupae. The three group treatments had significantly different conversion efficiencies of larvae to pupae (Poisson regression: $\chi^2 = 11.1$; df = 2; $p = 0.004$), with six-foundress groups having greater efficiency than three- and nine-queen groups (Z-test: $p < 0.05$), which did not differ from each other (Z-test: $p = 0.54$). All social groups had similar conversion efficiencies of pupae to workers (Poisson regression: $\chi^2 = 4.87$; df = 2; $p = 0.088$).

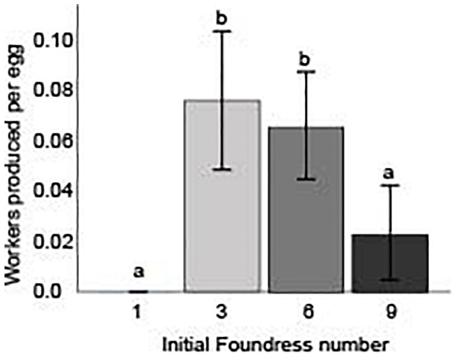

FIGURE 3 | Initial foundress number influenced the efficiency with which founding groups ultimately converted eggs into workers. The worker/egg ratio was calculated by taking the number of eclosed workers in the nest on day 50 and dividing that by the number of eggs in the nest on the day of peak egg production, day 9. Different letters above bars indicate significant differences between groups after *post hoc* analysis (Z-tests following negative binomial regression, $p < 0.05$).

Influence of Foundress Number on the Allocation of Labor

Queen activity budgets for different-sized foundress groups are shown in **Figure 4**. Foundress number significantly influenced the per capita performance of active behaviors, including walking, digging, foraging, and brood care (Negative binomial regression: $\chi^2 = 125.6$; df = 3; $p << 0.0001$, **Figure 5**). Solitary foundresses were significantly more active than foundresses in groups of three, six, or nine (Z-test; $p < 0.0001$). Furthermore, groups of three were significantly more active than larger groups (Z-test: $p < 0.0001$), but groups of six were not significantly more active than groups of nine (Z-test: $p = 0.135$).

Foundress number influenced the total performance of colony growth tasks within a nest in a different fashion than for overall activity (Negative binomial regression: $\chi^2 = 81.1$; df = 3; $p << 0.0001$; **Figure 6**). Solitary foundresses and groups of nine performed significantly fewer total colony growth tasks than groups founded by three or six foundresses (Z-tests: $p < 0.01$).

TABLE 4 | The conversion efficiency in brood development was estimated by using counts from the days of peak production.

Initial foundress number	Workers/egg	Larvae/egg	Pupae/larvae	Workers/pupae
1	NA	0.009 ± 0.009^{a}	NA	NA
3	0.08 ± 0.01^{a}	0.20 ± 0.02^{b}	0.29 ± 0.04^{a}	1.02 ± 0.16^{a}
6	0.07 ± 0.01^{a}	0.14 ± 0.02^{a,b}	0.46 ± 0.06^{b}	0.80 ± 0.15^{a}
9	0.02 ± 0.01^{b}	0.10 ± 0.01^{a,c}	0.27 ± 0.06^{a}	0.53 ± 0.15^{a}

Superscripts denote statistiacially significant results of Z-tests.

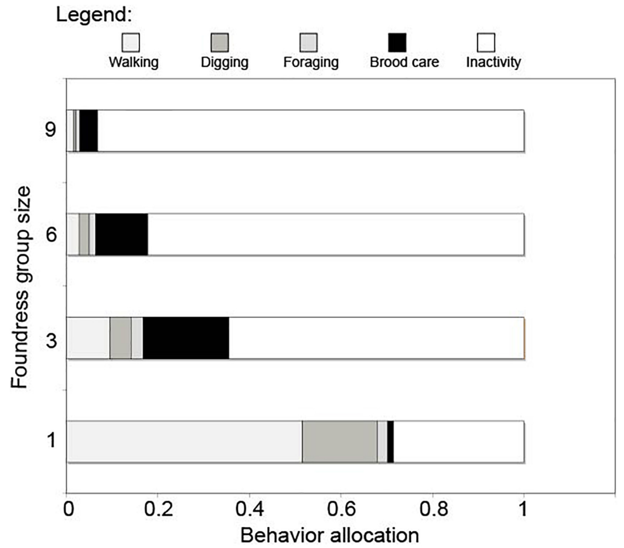

FIGURE 4 | The overall allocation of behavior in groups of foundresses. Behaviors were assigned to one of the 5 broad categories shown in the figure legend. The proportional activity of each queen was found by dividing the total of that category by the total number of observations across all five categories. This was averaged across nests and replicates. A broad pattern across behaviors can be seen where the overall activity levels decreased as foundress number increased.

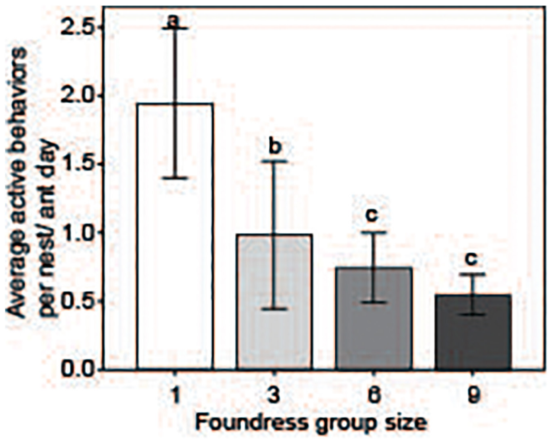

FIGURE 5 | Per-capita activity was assessed by summing the number of active behaviors performed by all ants in the nest (which included: Walking, grooming, excavating, foraging, as well as any other active engagement or behavior; see **Table 1**). This value was divided by the sum of the number of days each ant was alive and present in the study nest. The result was a metric of activity, the average behaviors performed per ant-day. Social groups of all sizes had significantly lower per-capita activity than groups founded by one queen. Different letters above bars indicate significant differences between groups after *post hoc* analysis (*Z*-tests following negative binomial regression, $p < 0.05$).

Groups of three and six did not differ from one another (*Z*-test: $p = 0.38$), but groups of nine performed significantly more colony growth tasks than solitary foundresses (*Z*-test: $p < 0.001$). The pattern was different when ant days were

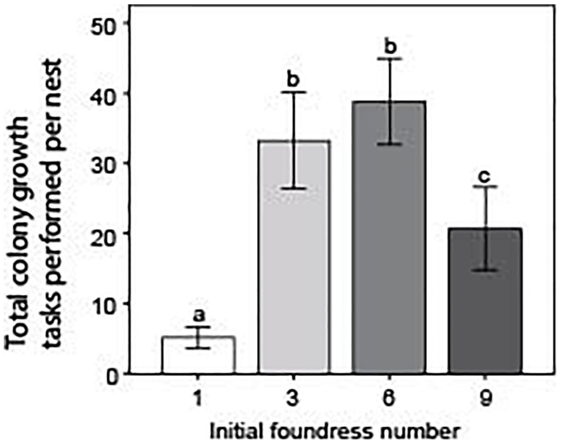

FIGURE 6 | Foundress number significantly influenced the total performance of colony growth tasks. Colony growth tasks included only tunneling, excavating, foraging, and tending brood. Without correcting for ant survival, the total number of tasks was summed up per nest and averaged across each treatment. Different letters above bars indicate significant differences between groups after *post hoc* analysis (*Z*-tests following negative binomial regression, $p < 0.05$).

taken into account. There was an overall significant difference across nest treatments (Negative binomial regression: $\chi^2 = 87.9$; df = 3; $p < < 0.0001$), such that per capita activity declined steadily with larger group size. Groups founded by one and three queens had the highest activity and did not significantly differ from one another (*Z*-test; $p = 0.47$). Groups of six queens performed significantly fewer tasks per capita than either one- or three-queen nests (*Z*-tests: $p < 0.001$); nine-queen groups performed significantly fewer than any of the other groups (*Z*-tests: $p < < 0.0001$).

Focusing even more specifically on brood care, foundress number significantly influenced the absolute number of brood-tending acts, with social groups far outstripping single-foundress nests (**Figure 7**). There were also some significant differences among social groups (Negative binomial regression: $\chi^2 = 9.0$; df = 2; $p = 0.011$). Groups of three and six queens performed the most brood care and did not differ from one another (*Z*-test: $p = 0.234$). Groups of nine performed significantly less brood care than groups of six (*Z*-test; $p = 0.002$) but did not significantly differ from groups of three (*Z*-test: $p = 0.056$).

Overall Fitness Metric

An overall fitness metric was calculated as the product of per capita worker production at day 50 and the proportion of foundresses surviving to day 50. This metric was 0.67 ± 0.10 for group size three, 0.57 ± 0.10 for group size six, and 0.16 ± 0.11 for group size nine ($\overline{X}$ *SD*). The three social groups differed significantly (ANOVA: $F = 6.24$; df = 2, 59; $p = 0.0035$), with groups of nine having significantly lower values than groups of three or six (Tukey tests: $p = 0.004$ and 0.022, respectively), which were similar to each other (Tukey test: $p = 0.744$). Because no queens or workers survived from the solitary foundress treatment, they were excluded from this calculation.

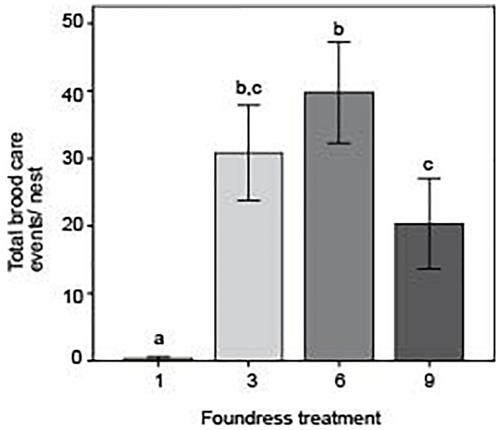

FIGURE 7 | Foundress number significantly influenced the absolute number of brood-tending events with groups of 3 and 6 caring most for their brood. Different letters above bars indicate significant differences between groups after *post hoc* analysis (Z-tests following negative binomial regression, $p < 0.05$).

DISCUSSION

Our results show strong concordance with observed typical queen numbers in field populations for the polygynous population of *Pogonomyrmex californicus*. Single queens had higher mortality—which suggests benefits of sociality. Interestingly, nests converged to the number of queens seen in the wild when they start in larger groups. All social groups were equally successful in producing workers, but intermediate groups were more efficient in their ability to convert eggs into workers. We were able to show that, mechanistically, this advantage was tied to increased efficiency at every brood transition stage (from egg to worker). This seems to have resulted from the allocation of work effort toward increased brood care at intermediate nest sizes. Out of the potential benefits of cooperative nest initiation, we found clear support for decreased queen mortality in groups and the ability for specific intermediate group sizes to produce the first workers more efficiently, but larger groups were ultimately similar to intermediate-sized ones in terms of queen survival and workers produced.

Enhanced Foundress Survival in Groups Regardless of Size

In our study, foundress number influenced survival—with foundresses in social groups surviving longer than solitary foundresses, regardless of group size. In several earlier studies of this population, a similar survival advantage was reported for groups of between two and six queens (Johnson, 2004; Clark and Fewell, 2014; Shaffer et al., 2016; Ostwald et al., 2021), along with some suggestions that larger group sizes can facilitate enhanced survival rates. By founding in groups, queens may enhance their own survival by sharing the work of colony foundation, which can involve costly and dangerous tasks and includes excavation, brood care, and foraging (Bernasconi and Strassmann, 1999). Foundress groups of *P. californicus* have been shown to develop a division of labor during colony foundation (Cahan and Fewell, 2004). This synergistic sharing of work may be responsible for survival benefits. For example, cooperative nest excavation may reduce water loss rate (Johnson and Gibbs, 2004) or allow allogrooming (Theis et al., 2015). Since the size of the group did not scale positively with individual queen survival probabilities in our nest context, survival benefits alone are unlikely to be the sole determining factor favoring group nest initiation for this species. If survival benefits were all that mattered, we would expect smaller group sizes (e.g., 2–3 queens) because of the longer-term reproductive advantage they would provide over the larger group sizes actually observed in the wild population. Thus, other advantages must be present as well.

Influence of Group Size on Brood Production and Efficiency of Worker Production

We found that, irrespective of founding group size, the timing of egg laying followed a similar trajectory. For example, all of the founding groups produced eggs during the first week of nest foundation and peaked in egg production on the same day, suggesting that individual physiological constraints limit egg production rates during nest initiation. Meanwhile, nests with social groups contained a similar number of eggs per queen, while solitary queens produced fewer—a result similar to that reported by Johnson (2004). While the groups of nine foundresses produced the most eggs, this initial advantage increasingly faded with each stage of metamorphosis. Where did the missing eggs go? Two non-mutually exclusive hypotheses are that some eggs may have succumbed to disease due to inadequate hygiene, or that eggs may have been eaten—as in trophic egg production (Brian and Rigby, 1978; Gobin and Ito, 2000; Perry and Roitberg, 2005).

Optimal Allocation of Labor at Intermediate Group Sizes

Foundress number influenced two key measures of fitness for incipient colonies: queen survival and the per-capita production of workers—suggesting an “optimal” intermediate group size. In addition to these measures, we more closely examined the allocation of labor and the efficiency of brood development in different-sized foundress groups, to allow us to pinpoint the mechanisms that determine optimal group size. Specifically, group size influenced both the overall activity levels of the groups as well as the allocation of labor. Small groups were most active, and as the founding groups grew larger, activity decreased. But while solitary queens were the most active of all, they performed fewer actual colony growth tasks than social groups. Solitary queens laid eggs, but the relative absence of brood care likely contributed to the increased failure rate of their brood to develop beyond the larval stage. Of all recorded behaviors, these solitary queens were most often observed walking (often at the surface of the sand in their observation nest). We speculate that these queens were searching—perhaps for other nest-founding partners. The Pine Valley population from which the queens were collected is primarily pleometrotic, hence these lone queens may be genetically programmed to search for founding groups to join. There are clear gene expression differences between queens found in predominantly pleometrotic Pine Valley relative to a nearby haplometrotic population (Helmkampf et al., 2016). A searching phenotype may be one manifestation of these genetic differences.

In contrast to the hyperactivity of the solitary queens, large founding groups (nine queens) were characterized by extreme inactivity. Even with an excess queen-ant labor force, fewer total colony growth tasks were performed in groups founded by nine queens relative to groups founded by three or six queens. Groups of nine also performed less brood care than groups founded by six. Thus, while these large foundress groups initially produced the largest number of eggs, the failure to perform colony growth tasks (including foraging, excavating, and brood care) likely contributed to the loss of this initial reproductive advantage.

Why were the largest groups so inactive? We have two hypotheses for why activity decreased as queen number increased. The first hypothesis relates to contact or interaction rate. A number of studies have shown that interaction rate may be an important cue that guides an individual social insect's response to changing colony conditions (Gordon and Mehdiabadi, 1999; Pratt et al., 2002; Smith et al., 2017). The high density of queens in groups of nine (and resulting increase in interactions) may have thereby triggered a shift in behavior in these queens from a state as active foundresses to a state as quiescent queens.

Our second hypothesis holds that foundress groups regulate their total amount of work to that needed to produce the colony's first cohort of workers. If we suppose that there is a finite amount of useful work that is necessary to produce this cohort, the inactivity of the large groups may make sense. Consider **Figure 4**—which shows the allocation of labor for an average queen in the different-sized groups. A queen in a group of three performed the most "useful" work. If there was a finite amount of work to be done we might predict that a queen in a group of six would have performed half as much work as a queen in a group of three. This is indeed what our data show. In turn, an average queen in a group of nine would have needed to do approximately 1/3 as much work as a queen in a group of three in this scenario, and again this is approximately what we see. In other words, the inactivity of the queens in the groups of nine may in fact be a rational response to the availability of work in the nest. With this perspective, the amount of work done by each queen may simply be an emergent social response to conditions in the nest. This could also be described as a kind of social homeostasis that influences the allocation of labor—where there is a finite amount of work to be done and a limit on the number of workers that a nascent colony can produce at that life history stage. While not being particularly efficient, the groups of nine queens got the basic job done: they survived and produced workers at the same rate in this early life history stage as the smaller social groups.

An Overall Metric of Fitness

By multiplying the per capita productivity of different foundress group sizes by the survival rate of those groups, we obtained a single estimate of fitness. With this standard, the intermediate-sized groups far outperformed the solitary queens and groups of nine. Thus, our results present a bit of a paradox. While all social groups performed equally well in terms of absolute numbers of brood production (especially the most important measure: workers), intermediate groups were more efficient in their ability to produce workers and consequently have a far greater cumulative fitness score.

The Socio-Ecological Context of Pleometrosis in *Pogonomyrmex californicus*

How do our results fit within what is already known of the social and ecological contexts of this and other species? Building upon work by Johnson, Overson reported an average of four queens in the population from which our queens were collected (Pine Valley; Johnson, 2004; Overson et al., 2014). This provides context for our finding that groups of three and six queens performed best in our study. The Pine Valley population probably represents a mixed evolutionarily stable strategy (ESS) where both cooperative and non-cooperative (aggressive) queens are present—but where cooperative (pleometrotic) queens are favored. The likely socio-ecological determinant is the high density and clustering of mature colonies found in Pine Valley—that results in intraspecific competition between clustered natal colonies (Shaffer et al., 2016; Haney and Fewell, 2018). In other species of pleometrotic ants, nest site limitation, highly clustered distribution, and densely located colonies seem to play a key ecological role in fostering cooperative nest founding (Tschinkel, 2006). Young colonies must likely contend with both predation from mature colonies as well as competition with other natal colonies. Our study captured only a narrow window at the beginning of the life of these colonies, but other work has demonstrated that the benefits of cooperative nest founding extend months into the early life of a colony, perhaps explaining why in addition to being pleometrotic, this population maintains primary polygyny in mature colonies, (Ostwald et al., 2021). In such contexts, a multilevel selection approach may be helpful in understanding the evolution of pleometrosis and related phenomena in social insect colonies (Rissing et al., 1989; Tsuji, 1995; Muir, 1996; Korb and Heinze, 2004; Reeve and Hölldobler, 2007; Dobata and Tsuji, 2013; Shaffer et al., 2016).

In spite of the artificial setting (ant farms), our study demonstrated clear fitness effects in terms of survival and production. This and similar ant farm studies are remarkable in this sense –young colonies (removed from the risks of predation and other environmental challenges found in nature) nonetheless demonstrate an influence on fitness and other emergent effects (such as changes in division of labor). This may make sense if we consider that in nature each incipient nest is a microcosm where a young queen (or queens) is generally inactive and stays within the nest as much as possible (besides necessary foraging trips). Most of the real "action" (and necessary work) occurs within the confines of the nest. But there may yet be unaccounted for environmental effects for young colonies in natural settings. Further field-based studies will hopefully shed further light on the adaptive value of pleometrosis. Regardless, our paper shows that selective pressures inside the natal nest favor cooperative group formation. However, such cooperation has limitations that create size constraints under which grouping is truly adaptive.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ants were collected with permission of the residents of Pine Valley, California. All ant queens were handled with an eye toward maximizing their survival during collection. Ants were handled according to current laws of animal welfare regulation, and as invertebrates—no institutional approval was required. No ant was deliberately harmed in the course of this study.

AUTHOR CONTRIBUTIONS

ZS, RC, and SD carried out the experiment. ZS, SP, and SD analyzed the results. ZS wrote the first draft of the manuscript. ZS, RC, SP, and JF designed the experiment and prepared the manuscript. All authors contributed to the article and approved the submitted version.

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Dispersal Risks and Decisions Shape How Non-kin Groups Form in a Tropical Silk-Sharing Webspinner (Insecta: Embioptera)

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Relying on silk can promote sharing, especially when its presence means life and its absence, quick death. In the case of Embioptera, they construct silken tubes and coverings exposed on tree bark in humid and warm environments or in leaf litter and underground in dry habitats. These coverings protect occupants from rain and natural enemies. Of note, adult females are neotenous, wingless and must walk to disperse. Evidence is pulled together from two sources to explore mechanisms that promote the establishment of non-kin groups that typify the neotropical *Antipaluria urichi* (Clothodidae): (1) a review of relevant information from 40 years of research to identify potential drivers of the facultative colonial system and (2) experimental and observational data exploring how dispersal contributes to group formation. To determine risks of dispersal and decisions of where to settle, adult females were released into the field and their ability to survive in the face of likely predation was monitored. Additional captured dispersers were released onto bark containing silk galleries; their decision to join the silk or to settle was noted. An experiment tested which attributes of trees attract a disperser: vertical or horizontal boles in one test and small, medium, or large boles in another. While walking, experimentally released adult female dispersers experienced a risk of being killed of approximately 25%. Dispersers orient to large diameter trees and join silk of others if encountered. These results align with observations of natural colonies in that adults and late-stage nymphs join existing colonies of non-kin. Experiments further demonstrated that dispersing females orient to vertical and larger diameter tree-like objects, a behavior that matched the distribution of field colonies. The ultimate reason for the observed dispersion pattern is probably because large trees support more expansive epiphytic algae and lichens (the food for this species), although the impact of food resources on dispersion has not been tested. Finally, further research questions and other webspinner species (including parthenogenetic ones) that warrant a closer look are described. Given that this group of primitively social insects, with approximately 1,000 species known, has remained virtually unstudied, one hope is that this report can encourage more exploration.

Keywords: silk spinning, social behavior, predator-prey interactions, egg parasitism, habitat selection

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INTRODUCTION

For a diversity of insects and spiders, ecological factors that can influence differential success of individuals living a solitary life or as part of a group include the costs and risks of dispersal, pressures imposed by predators and parasites, and competition for resources (reviewed in Choe and Crespi, 1997; Costa, 2006). "Basic necessary resources," *sensu* Choe and Crespi (1997), include silks because for many insects and spiders these proteins form critical defenses. Despite the value of shared silk, solitary individuals might do well to disperse to seek uncontested food or to avoid parasites that might have built up in the colony. Solitary silk spinners often can quickly construct a new domicile. But are they better off? Sharing the tasks of spinning and silk production and the more extensive silk structure produced by a group might be a better strategy. Possible reasons for remaining part of a colony include greater protection from predators, more efficient prey capture if silk is used as a snare, taking advantage of the spinning by colony-mates and/or avoiding the costs and risks of dispersal. In pholcid spiders, for example, spiderlings grow more quickly and attain larger sizes when they share silk spun by larger conspecifics in the colony (Jakob, 1991). The tendency to share silk by social spiders of the genus *Stegodyphus* helps to protect individuals from predators as well as more efficiently traps prey (Seibt and Wickler, 1990). A detailed field study of *S. dumicola* in Namibia showed that arboreal ants could be held at bay by social spiders capable of producing copious sticky silk (Henschel, 1998). However, solitary spiders in that population were better able to avoid a contagious fungal disease that more easily spread in large colonies. Apparently, *S. dumicola* exists in solitary and social groups because of fluctuating costs and benefits of dispersing vs. remaining in their natal group to share silk abodes. Dispersal tendencies and sociality levels in spiders have also been shown to correlate in an investigation of seven species of *Anelosimus* (Corcobado et al., 2012). By analyzing morphological traits, the authors found that higher degrees of sociality were negatively correlated with the tendency and ability to disperse.

Populations composed of solitary and colonial reproductives typify another silk-sharing arthropod, insects of the little-known order Embioptera (a.k.a. Embiidina and Embiodea), commonly known as webspinners. The purpose of this report is to identify factors related to the establishment of non-kin groups, the previously demonstrated structure for the neotropical webspinner *Antipaluria urichi* (Saussure) (Family Clothodidae)—the subject of numerous laboratory and field experiments and censuses (reviewed in Edgerly, 1997, 2018). For this species, evidence points to colony structures that vary from solitary females with their offspring to groups of non-kin adult females that share contiguous silk coverings (Edgerly, 1987a). Field collections of webspinner species over a hundred years demonstrated that some of the estimated 1,500–2,000 species (mostly undescribed, Miller et al., 2012) form colonies of adult females with their young (e.g., Melander, 1903; Bradoo, 1967; Edgerly et al., 2002, and reviewed in Edgerly, 1997; Costa, 2006), whilst others live as single mother-offspring groupings (Edgerly et al., 2002). Indeed, in some species, solitary females are quite aggressive toward others and do not exhibit group-living (Ross, 2000a,b). The phylogenetic pattern and ecological correlates of these different social groupings are not known. This report is multi-faceted, describing basic biology and filling in gaps in knowledge, as follows: (1) a review of the natural history of *A. urichi* and of research findings related to the cost and benefits of group living, (2) presentation of methods and results of experiments on dispersal behavior of adult females, especially relevant to the question of how non-kin groups form, (3) an analysis of the risk of predation for solitary and colonial adult females, and (4) discussion of avenues for further research to address why *A. urichi* disperse from their natal colonies and why they often join non-kin groups rather than settle as solitary females raising their young. Lastly, given the lack of knowledge of embiopteran colony structure throughout the range of this cosmopolitan order, a brief summary of lifestyle diversity for webspinners, needed future studies to address missing information, and comparison to other silk-sharing arthropod species will be presented.

For embiopterans, silk functions as protective armor and shapes their lives—morphologically and behaviorally. Sharing silk may have promoted social behaviors in some species who gain an advantage from more expansive coverings. From arboreal bark-dwellers to subterranean crevice-dwellers, webspinners all have the same body shape and basic lifestyle (**Figure 1**)—a very different scenario when compared to the more hyper-diverse taxa in the Class Insecta. Nymphs and adults alike spin by stepping with their front legs, while releasing dozens of nano-scale silk fibers from modified hair-like ejectors that clothe the bottom of their front tarsi (Büsse et al., 2019). They execute

FIGURE 1 | Portraits of *Antipaluria urichi*. **(A)** Adult male (winged individual, 1.2 cm long) and female (1.5 cm long) in a lab culture container of dried Live Oak leaves and silk; **(B)** adult females from five taxonomic families, displaying typical uniform shape and a range of colors (body length not to scale). Families from left to right: Australembiidae, Archembiidae, Embiidae, Oligotomidae, Clothodidae.

elaborate spin-steps to fashion tubular galleries and sheets of tissue-like waterproof coverings (Osborn Popp et al., 2016; Stokes et al., 2018; Harper et al., 2021) that protect them from rain and predators (**Figures 2A–E**). Adult females are neotenous (**Figures 1, 2F**), flexible, and soft-bodied whilst adult males, usually winged and short-lived, sport wings that fold up so they can run backward and forward inside the silk without getting tangled (Ross, 2000a). Embiopterans are also known for the order-wide occurrence of maternal behavior (Ross, 2000b). Adult females vary in how they handle and protect their eggs, but generally, they cluster them—often with elaborate coverings of macerated materials and silk that can act as a shield against the threat of parasitism—and guard them (Edgerly, 1987a,b).

Between 1982 and the present day, I along with numerous co-authors have conducted experiments and censused field colonies of *A. urichi* to determine the extent of their silk coverings and number of occupants (Edgerly, 1987a,b, 1994), types of trees that support them (Edgerly, 1987c), environmental correlates that predict their presence and abundance (Shenoy et al., 2020), cost of spinning silk (Edgerly et al., 2006), how they spin (Edgerly et al., 2002, 2020; Büsse et al., 2015, 2019; McMillan et al., 2016), as well as attributes of their silk and how it acts as waterproofing (Osborn Popp et al., 2016; Stokes et al., 2018; Shenoy et al., 2020; Harper et al., 2021). Related to the occurrence of non-kin groups, the first question posed for my Ph.D. work, under the guidance of the late George Eickwort of Cornell University, was basic: "what type of social behavior do webspinners exhibit?" Before that time, no one had conducted quantitative field experiments, although several observational studies converged on the characterization that embiopterans exhibited maternal care of young (reviewed in Edgerly, 1997; Costa, 2006; Edgerly, 2018). Additionally, evidence of expansive colonies in the more humid tropical regions was especially intriguing (Ross, 1970). Trinidad's field station, Simla, was the chosen site for my dissertation work because of reports that arboreal colonies of *A. urichi* (at that time, known as *Clothoda urichi*) were abundant and easy to find.

Natural History of *Antipaluria urichi*

As informed by field censuses, *A. urichi* is characterized as facultatively colonial (Edgerly, 1987a; see below for more details). They feed on epiphytic lichens and algae on vertical surfaces, including large outdoor plant pots, cement walls, road banks and especially trees of a variety of species (Edgerly, 1987c). They continually extend their tissue-like silk to cover new feeding sites during nocturnal activity bouts (Edgerly, 1987b). Adult males are winged (**Figure 1A**) and they fly from their galleries in the afternoons in search of females (personal observation). The male's antennae are long and covered with chemoreceptors and pheromonal communication is likely although not investigated for any webspinner species. Males do not feed after reaching maturity and die soon after mating. Females produce an elaborate egg mass averaging 53 eggs (Edgerly, 1987b). They coat each egg

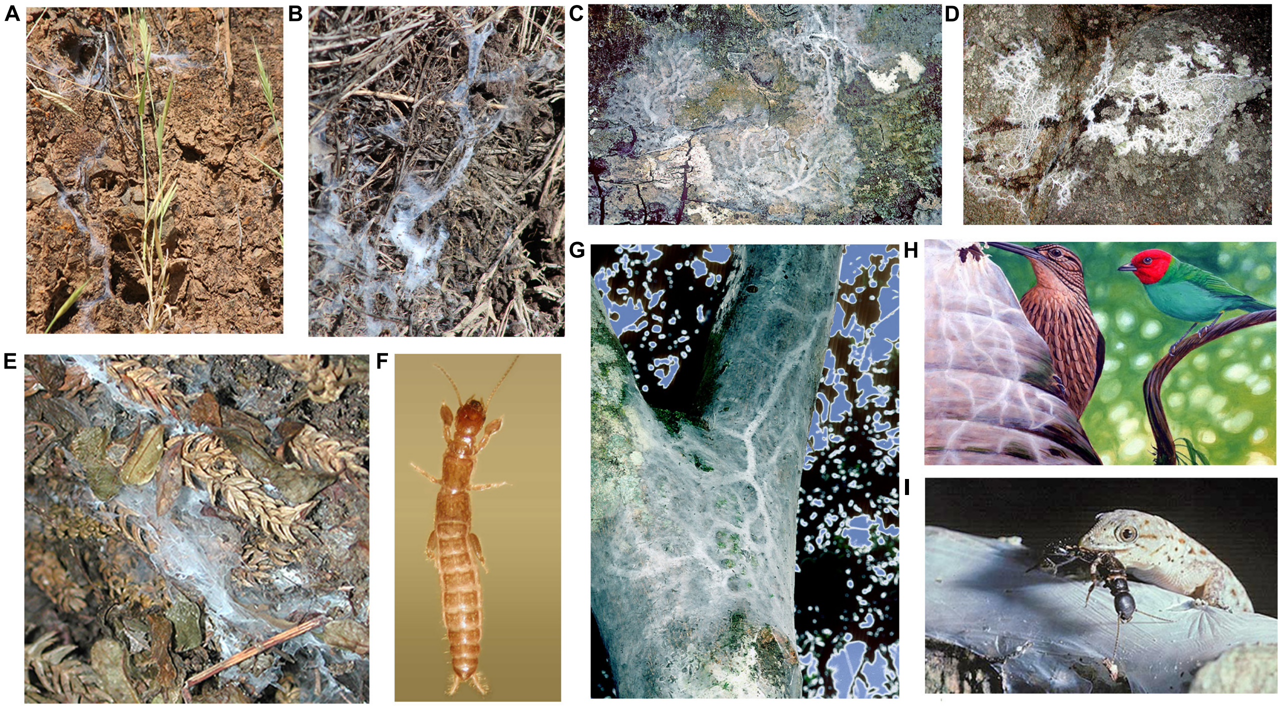

FIGURE 2 | Variation in silk domiciles and habitat for webspinners and potential predators of *Antipaluria urichi* of Trinidad. **(A)** *Haploembia tarsalis* and **(B)** *H. solieri* silk in California. **(C)** *Pararhagadochir trinitatis* silk in Trinidad. **(D)** *Notoligotoma hardyi* silk on granite outcrop on Magnetic Island. **(E)** *Metoligotoma incompta* silk on Magnetic Island, Queensland. **(F)** *H. tarsalis* adult female (1.0 cm in length). **(G)** *A. urichi* silk in Trinidad showing thick silk typical of an egg-guarding female. **(H)** Bay-headed Tanager and Woodcreeper, two birds seen tearing into *Antipaluria urichi* silk. **(I)** Gecko preying upon adult female of *A. urichi* caught outside of her silk. For scale, the webspinner is approximately 1.7 cm in length. Photographs by the author; painting of bird predation by Edward C. Rooks.

with a cement-like material, sticking the eggs to the substrate in organized rows, covering the clustered eggs with gathered, macerated materials and silk, and topping the whole structure with thick silk (Edgerly, 1987b). As demonstrated by detailed time budgets, the mothers stand guard for approximately 6 weeks, helping to prevent egg parasitism by ubiquitous scelionid wasps (*Embidobia urichi* Ashmead) (Edgerly, 1987b). If the webspinner mother is not present, the wasps can dig through the egg mass covering (0.16 cm thick on average) to reach and parasitize the eggs. Maternal care is necessary to keep the silk intact to avoid parasitism by the wasps and predation by ants of the eggs (Edgerly, 1987b). The outer silk of a domicile constructed by egg-guarding females is relatively thick (**Figure 2G**). Nymphs rely on their mother's production of silk, which increases exponentially in area after the eggs hatch because of her emphasis on spinning (Edgerly, 1987b, 1988). The young grow faster in her presence (Edgerly, 1988), a phenomenon also known from other webspinner species (Rita, 1993; Choe, 1994). A laboratory experiment on the cost of silk also showed that nymphs attained smaller size as adults on average if they were repeatedly forced to replace stolen silk (Edgerly et al., 2006). A European species (*Embia ramburi*) is known to collect and retrieve food pieces for their young (LeDoux, 1958) but *A. urichi* does not display this more elaborate maternal investment. In fact, their colonies deplete food resources under the cover of silk, which is extended to enclose more and more food as the nymphs develop. Central-place foraging habits displayed by other social insects is not adopted by *A. urichi* and as will be proposed below, may partly explain their dispersal behavior, a focal topic for this report.

The silk of *A. urichi* is a diffuse cover over resting and foraging sites. These characteristics reflect the point made by Choe and Crespi (1997) in their review of silk-sharing species—that is, individuals living in such a diffuse domicile cannot easily monitor or dominate others in the group. In fact, colonial females share silk but do not appear to cooperate in other ways. Silk coverings found at one field site in the Northern Range Mts. of Trinidad varied from 11 cm^2 for a solitary female to 37,000 cm^2 for colonial females. One large colony contained 72 individuals, with 24 being adult females. At one location, a survey of 44 colonies found 86% of 138 adult females shared silk with others. While at two other sites, 37% of 57 (Edgerly, 1987a) and 60% of 35 (Edgerly, 1994) adult females were colonial, indicating the commonality and variability of the facultatively colonial structure. Individual colonial females lay significantly more eggs than do solitary females (Edgerly, 1987a). The underlying cause of the difference in the number of eggs laid by solitary and by colonial females is not known. Laboratory experiments on the cost of spinning showed that the number of eggs laid by females that had their silk repeatedly stolen, necessitating replacement, did not differ significantly from those laid by controls (Edgerly et al., 2006). Despite being able to produce more eggs in field colonies, colonial females experience higher rates of egg parasitism and in the end, the number of first instars that emerge do not differ significantly between solitary and colonial females (Edgerly, 1987a). Even though the question of proximate causation remains, higher egg counts appear as a potential benefit and higher parasitism rates appear as a potential cost of group-living.

Webspinner species differ in how much silk they produce in the field and in the lab, even during the short term. One hypothesis is that arboreal species that live exposed on bark surfaces in rainy tropical regions require more silk as cover than those that live in dry habitats under rocks and in leaf litter where substrate materials are stitched together to form protective domiciles (Edgerly et al., 2006). Requiring more silk would be a reason to join others, so that the silk production and spinning task can be shared. Indeed, laboratory experiments comparing silk production and gregarious tendencies for the arboreal *A. urichi* and for the litter dwelling *Metoligotoma incompta* (Ross) (previously *Australembia incompta*; Family Australembiidae) revealed that the arboreal species produced significantly more silk in a short amount of time (Edgerly et al., 2006). Furthermore, when allowed to disperse one at a time into mock habitats of bark and lichens, *A. urichi* females settled into a contagious dispersion pattern, with an average distance between females of one body length. The litter-dwellers did not spin any silk when placed in petri dishes during a 3-day trial, unless a leaflike object was provided. They also spread out randomly rather than settle near each other when allowed to disperse into a mock habitat. They averaged 1.7 body lengths away from each other and did not share silk. These results are limited in scope because differences in spinning behavior might be due to other underlying conditions, such as differences in food sources, silk proteins and the like. Given the availability of a phylogeny (Miller et al., 2012) and our knowledge of many more species that vary in silk spinning tendencies, a range of species can now be studied to address a similar research question in the future. Nonetheless, *A. urichi* is a spinner that produces copious silk and is likely to settle near another individual when given the chance. The laboratory results align with the field observations noted above, and with the behaviors described below.

Previously published census work established that natal dispersal is a regular feature of *A. urichi* life cycle. The occupants of 64 colonies, defined as distinct patches of silk and marked with flagging tape around the field station at Simla, were monitored for residents (including individually marked adult females), presence of eggs, hatch rates, parasitism, evidence of predation and so forth in an area approximately 1,400 m^2 (Edgerly, 1987a,b, 1988). During the field season between September and December 1983, 16 unmarked adult females and 10 late-stage nymphs migrated in from elsewhere and settled into the flagged colonies (Edgerly, 1988). New silk galleries also appeared on trees near the previously flagged colonies: 31 established by single adult females and nine by solitary late-stage nymphs. By the time their offspring were approximately half grown, none of the mothers were still present (Edgerly, 1988). Reproductive females with eggs or nymphs were found dead in the silk (n = 3), disappeared for unknown reasons (n = 25), or after predator attacks either by ants or after large holes were torn in the silk above their resting sites (n = 16) (see Table 2 in Edgerly, 1988). They do not display overlapping generations nor have females been seen to lay more than one batch of eggs. Previous work on maternal care of eggs and nymphs (Edgerly, 1987b, 1988) supported the conclusion that a female lays one batch of eggs, spends 6 weeks protecting them, and then remains with her nymphs until she dies or disappears. No instance of repeated egg-laying has been observed for this species but the question remains whether it is possible. During the field census, the number and development of nymphs in fifteen colonies that could be closely monitored was recorded. The nymphs slowly disappeared over time, until only two colonies produced adult females *in situ* (2 in one and 11 in the other) (see Figure 1 in Edgerly, 1988). The regular disappearance of nymphs from their natal galleries and the mere appearance of new silk galleries reflects the potentially high rate of natal dispersal by *A. urichi.*

Risks of Predation for Solitary and Colonial Females

Predators, birds and especially ants, cut into silk and kill or catch and carry away their embiopteran prey (Edgerly, 1988, 1994; **Figures 2H,I**). Because silk functions as a shield for the soft-bodied insects inside, exploring how cuts and tears in the silk accumulate can be used as a measure of predation risk. The two-dimensional silk covering of *A. urichi* is conspicuous, the area of a colony is easily quantified, and the occupants can be counted. To determine predation rate as a function of silk area and individuals within, I spent 3 weeks monitoring 47 colonies in the rainforest at the Asa Wright Nature Centre in Trinidad, documenting in detailed drawings the cut and torn holes as they accumulated (Edgerly, 1994). Even if holes were ultimately patched by the occupants, scars were still discernible. My assumption was that at least some of these holes reflected the predation attempts like those previously witnessed. At that time, I was interested in whether larger silk expanses attracted more attention than smaller patches to test the encounter effect hypothesis (*sensu* Turner and Pitcher, 1986). The prediction was that larger silk patches accumulate relatively fewer attacks based on area alone because they are not proportionally more attractive than smaller patches. Most ants will walk over the silk as if it were part of the substrate and not as if it were possible prey, but others do cut in as mentioned. I found that cut holes accumulated less than would be expected based solely on the perimeter of the silk covering. I also discerned that a measure of risk (number of holes per individual per silk perimeter) varied greatly, with smaller colonies experiencing the greatest variability in potential risk per individual: both the lowest and the highest risk levels were in this category. I concluded that individuals dwelling under smaller patches of silk, as is true for solitary females, would experience unpredictable and sometimes very high levels of risk from predators. What I did not test at the time was whether solitary adult females suffered greater risk of attacks than those living in colonies. Because adult females are mostly responsible for spinning and contributing to the expansion of silk, their presence matters more than the total count that includes nymphs. To address the question of why dispersing adult females join others, I re-analyzed the data from Edgerly (1994) for this report to examine how much risk adult females might experience. I sorted them into three groups: solitary adult females, solitary mothers with nymphs, and colonial adult females, which ranged from two to seven at the field site. The reason solitary females with nymphs are considered as a separate group is because once the eggs hatch, the mother expands her silk area exponentially compared to the time she is guarding her eggs (Edgerly, 1988). My prediction is that solitary females (without nymphs) will experience the greatest variability in risk and have less silk on average. They might be completely missed by hunting predators because the silk patch is small. But if they are attacked, the higher risk level (the holes per individual per silk expanse) means they likely will be killed.

This report emphasizes field surveys and natural history to establish context as well as experimental work and quantitative observations. A major intent is to share knowledge of this little-known order of insects while also addressing questions about the dynamics of colony structure. Experiments on the behavior of dispersers and associated risks, described in Edgerly (1987c), have not previously been published. Because of the relevance to how non-kin groups form, the methods and results are presented below.

MATERIALS AND METHODS

Dispersal Behavior and Non-kin Group Formation

Field Experiment 1: To Join or Not to Join?

To observe the process of dispersal, five naturally dispersing adult females were caught walking in the open in the field many kilometers away from Simla and brought back to the field station. Over 2 days, four trials for each female were conducted; each female was allowed to walk out of a petri dish and onto the base of a tree trunk that supported small silk galleries occupied by at least one resident individual *A. urichi.* The actual number of occupants was not determined because opening the silk to count them would have disturbed the silk. The experiment revealed if dispersers try to settle in already spun silk or if they settle in a spot to spin their own domiciles. Trials were ended when the disperser entered the silk but then left the silk completely (scored as “not joined”) or when agonistic interactions between resident or the disperser stopped for at least 5 min and the disperser remained sitting still in the silk (scored as “joined”). After each trial, the dispersers were collected and returned to their petri dishes. Each female was tested again on a different tree.

Field Experiment 2: Risks for Dispersers

Dispersal behavior by these wingless soft-bodied insects without any obvious defenses, except to run fast backward (a unique ability of webspinners), appears particularly risky. To determine how risky, pre-reproductive adult females ($n = 33$) were released on the ground at the field station and followed. Their fate was recorded as “killed by predator,” lost from view while walking, or settled onto a tree either alone or having joined another’s silk.

Laboratory Experiment: Habitat Selection Explains Dispersion

To investigate behavior of dispersers in a controlled environment, pre-reproductive adult females ($n = 20$) were collected in the field, held in petri dishes, and fed for 2 days prior to testing. Their choice of a tree-like habitat was tested in a 9 m^2 indoor space wrapped with white cloth to create an arena with diffuse lighting. A hole in the cloth provided viewing by a hidden observer. A vertical stick (1 cm diameter, 30 cm tall) was affixed in the center of the arena and the test objects providing mock habitats for dispersers were arrayed around the arena edges. In the first test, one vertical and four horizontal logs (9 cm diameter, 1 m long) were placed in the corners, evenly spaced and 1 m away from the center vertical stick. The logs were rotated clockwise after each trial. For each trial, a female was released into the center of the arena near the vertical stick. This method was employed because when dispersers were released during field trials, they climbed up a nearby object (such as a vertical grass stem) and turned their heads back and forth before setting off toward a nearby tree. The central vertical stick provided such a climbing post for the dispersers; they all climbed it when released into the arena. In the second experiment, females (n = 15) were given the choice of three differently sized models of vertical logs, made of brown paper (diameters of 7, 11, and 30 cm), again rotated between trials, and placed 1 m away from the central stick. This experiment was replicated three times with the same females tested in random order but without individual identification. A Chi-squared Goodness of Fit test was employed for each trial, with a null hypothesis of random distribution relative to the three choices in each trial and a significance level of $P < 0.05$. For this and other statistical analyses, JMP Pro 16 software was used by SAS Institute (2021). To gain an understanding of natural dispersion, the presence and absence of colonies on all trees equal to or larger than 10 cm diameter at breast height (DBH—bole diameter measured at approximately 1.4 m from the ground; Cooperrider et al., 1986) was recorded at another site, an abandoned tonka bean plantation on the Arima-Blanchisseuse Road near the Asa Wright Nature Centre.

Risks of Predation for Solitary and Colonial Females

Relying on data from Edgerly (1994), I conducted non-parametric analysis of variance (Kruskal-Wallis test) to determine if predation risk differed between three types of colonies: solitary adult females (n = 7), solitary adult females with nymphs (n = 7), and colonies with grouped adult females with or without nymphs [n = 6; mean number of adult females = 3.5 ± 0.85 (SE)]. Predation risk was measured as the number of holes cut/perimeter of silk/number of individuals in the silk that accumulated over 3 weeks. Area of silk per colony sorted in the three groupings based on occupants was also compared.

RESULTS

Field Experiment 1: To Join or Not to Join

All five released dispersers entered a naturally occurring silk domicile that they discovered while walking up the tree bark. Four of the dispersers joined the occupant of a silk domicile for three trials but did not join in one trial (entered but quickly left). The fifth disperser joined the discovered silk domicile in all four trials. In 14 of the 20 trials the resident of the domicile responded with vigorous shaking as the disperser entered the silk. The agonistic interactions were usually not enough to push out intruders; those dispersers that joined moved away from the resident to settle a short distance away but still within the now-shared silk covering.

Field Experiment 2: Risks for Dispersers

Dispersing females released in the field initially climbed onto a vertical stem or protruding leaf litter and turned their heads back and forth, appearing to scan the surroundings. They then dropped off their perches and walked directly to large vertical shapes. In an open grassy area near the field station buildings, they (n = 12) walked quickly at approximately 16 cm per minute for about 6 m toward towering objects: the field houses or a large stand of mahogany trees. Six were killed before reaching their destinations. In the forest, they (n = 21) moved at 20 cm per minute. They walked an average of 3 m before climbing large trees; two were killed on the ground. Of the 19 tree climbers, I was able to follow nine, losing sight of the others; four entered pre-existing silk and five settled on bark to spin on their own. This result reflects the distribution of females in the field: solitary and colonial females exist in the same population. Tree climbers regularly nibbled the surface of the bark, appearing to sample the quality of the habitat. In summary, mortality due to predation for 33 walking females was at least 24% (six by ponerine ants and two by wolf spiders), reflecting the riskiness of walking in the open.

Laboratory Experiment: Habitat Selection Explains Dispersion

In all trials, females climbed to the highest point of the central stick, turned their heads scanning the room, returned to the floor, and walked directly to one of the objects. In 17 of 20 log orientation trials, females chose the vertical instead of the horizontal logs (Goodness of Fit Test X^2 = 38.4; DF = 2; $P < 0.005$) and position in the room did not matter (Goodness of Fit Test X^2 = 0.4; DF = 2; P = 0.5). In the test of size preference, females overwhelmingly preferred the largest log in two trials but split between large and medium sizes in the third trial (**Figure 3**). The colony dispersion pattern at the field census site again reflected a preference for recruitment to and success on large trees (**Figure 4**).

Predation Risks for Solitary and Colonial Females

Silk coverings for adult females differed in area, with solitary females covered by less silk area than grouped females (Kruskal-Wallis X^2 = 10.423; DF = 2; P = 0.005; **Figure 5A**). The finding that some solitary mothers with nymphs displayed more expansive silk than did egg-guarding mothers reflects their behavior of dramatically increasing silk production after their eggs hatch (Edgerly, 1988). The median risk of predation (number of holes per perimeter of silk per individual) did not vary between solitary, solitary with nymphs or colonial adult females (Krusal-Wallis Test: X^2 = 0.7685; DF = 2; P = 0.6810). The variances for risk were significantly different, with solitary females showing the greatest variation (Bartlett's test: F = 16.1006; DF = 2; $P < 0.0001$; **Figure 5B**).

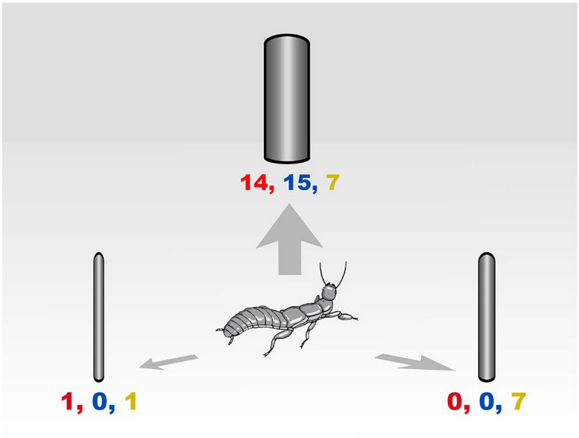

FIGURE 3 | Dispersal choices by adult female *Antipaluria urichi* in the laboratory. Paper cylinders represent trees of three sizes: 7, 11, and 15 cm diameter and 1 m tall. Each tree model was 1 m away from a central stick placed where the disperser would climb to survey the scene. Relative positions are not to scale in the drawing. Numbers represent the number of females choosing each cylinder in the choice test. Trial 1 results shown in red letters (Goodness of Fit Test $X^2 = 30$; $DF = 2$; $P < 0.0001$; Trial 2 in blue letters: Goodness of Fit Test $X^2 = 24.4$; $DF = 2$; $P < 0.0001$) and Trial 3 in orange letters (Goodness of Fit Test $X^2 = 4.8$; $DF = 2$; $P = 0.09$).

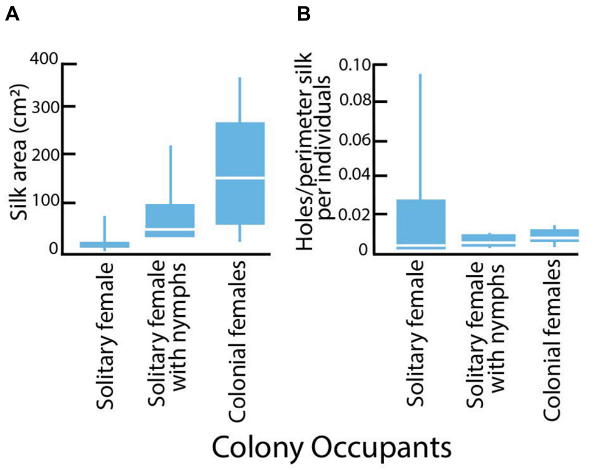

FIGURE 5 | Silk colony sizes and risk of predation for field colonies of *Antipaluria urichi*. Box plots show medians, quartiles and ranges. Sample sizes: solitary adult females ($n = 7$), solitary females with nymphs ($n = 7$), and grouped females ($n = 6$). **(A)** Area of silk expanse (cm^2) for adult females in the three different colony types. **(B)** Risk of predation as measured by accumulation of cuts per perimeter of silk covering per individual recorded over 3 weeks in field colonies in the Northern Range Mts of Trinidad.

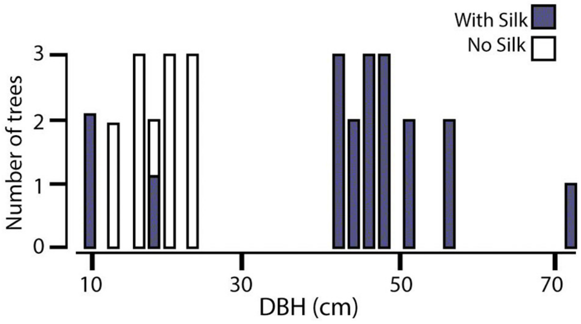

FIGURE 4 | Colony dispersion on trees of different sizes in an abandoned Tonkabean plantation in the Northern Range Mts of Trinidad. DBH, diameter at breast height.

DISCUSSION

Dispersal Behavior Partially Explains Dispersion

Dispersal behavior is a focus of research on social behavior because of its role in shaping how groups form and/or if they dissolve. For example, when sexually mature offspring delay dispersal and remain in their natal nest or colony, helping behaviors have evolved in a variety of social animals including birds (Koenig and Dickinson, 2016), ambrosia beetles (Nuotclà et al., 2021), small carpenter bees (Rehan et al., 2014) and social spiders (Henschel, 1998) to name a few. These examples represent groups of kin that cooperate at some level, which contrasts *A. urichi* colonies that vary from solitary mother-offspring groups to colonial non-kin reproductives that share silk domiciles. My detailed field censuses early on showed that dispersal out of the natal colony is a feature of the life cycle of *A. urichi* and that aggregations are not composed of overlapping generations of kin (Edgerly, 1988). The reasons that *A. urichi* disperse must be critical for survival because walking outside of their silk covering is very risky; the reasons they join others to share silk when they do disperse still needs to be resolved.

Dispersing *A. urichi* tend to walk to large trees and in their rainforest habitat, these trees often support conspecifics, as reflected in the dispersion of colonies of trees shown in **Figure 4**. If a disperser encounters silk, she will enter it. This behavior can lead to the establishment of non-kin groups if the disperser settles there. Evidence revealed by tracking dispersers in the field suggests that the risk of being outside of silk is high enough that entering silk upon its discovery promotes survival. When experimental dispersers chose to enter another's domicile residents responded aggressively. Detailed laboratory experiments examining how residents respond to intruders (Dejan et al., 2013) showed that resident adult females signal with various shaking movements when individuals attempt to join their silk. The same response by residents was observed for the field disperser experiment described above. In the 2013 lab experiment, individual pre-reproductive females shook when an intruder entered their silk but quickly stopped signaling and allowed the intruder to settle next to her. Egg-guarding females, in contrast, displayed more vigorous, numerous and varied signals (lunge, shake, snapback, and push-up) directed toward female intruders (nymphs and adults alike) (see Figure 5 in Dejan et al., 2013). Despite the more aggressive responses by these egg-guarders, intruders tended to settle in the silk covering but would stay a short distance from the resident female thus avoiding triggering her aggressive responses. Of interest is the fact that an adult male that entered the silk of an egg-guarder triggered signals but once the male contacted the female she stopped signaling. Females were much more reactive when the intruder was a female.

If one considers the behavioral repertoire just described, it appears that *A. urichi* females join silk structures for immediate safety. They do not appear to cooperate with the residents except to extend the existing silk structure for their own purposes. Why are residents aggressive when dispersers enter their silk? A possible underlying selective pressure includes higher egg parasitism rates when other nearby egg-layers share silk (Edgerly, 1987a). Untested possibilities include competition for food and risk of cannibalism of eggs and perhaps of vulnerable newly molted nymphs (Edgerly, 1987c). It is not known if colony-mates represent a threat, but as hypothesized by Ross (2000b), cannibalism remains as a possible reason why even the nymphs can be aggressive toward non-kin dispersers that entered their silk abodes.

When silk spinners join together, one might expect that the group would gain an advantage from numbers alone. As described in reviews of the evolution of social behavior (e.g., Costa, 2006; Taborsky et al., 2021), augmented groups can benefit for a variety of reasons including shared vigilance, territory defense or thermoregulation. More adult webspinners in a group can mean a greater expanse of silk, as shown in **Figure 5A** and in previous reports from field censuses (e.g., Edgerly, 1987a, 1994). The hypothesis that grouped adult females do better against predation threats has support only if high variability in the intensity of predator attacks on silk matters, because the median attack rate did not differ for solitary and grouped adult females. Other hypotheses to explain why non-kin groups form remain to be tested, as follows: (1) Females might join others and stay to oviposit because the food is better in particular spots, possibly explaining the higher egg counts for colonial females detected in the field colonies (Edgerly, 1987a). (2) Females join others because more extensive silk protects better against the elements, like heavy tropical rains. Grouped females might be able to recover more quickly after the silk is damaged as has been shown for social spiders that live in larger groups in their rain forest environment (Hoffman and Avilés, 2017). (3) Females join others because their nymphs have a better chance when the silk is more extensive, especially if the mother dies early in their development. Given that nymphs grew more slowly without their mother in a field experiment (Edgerly, 1988) and nymphs achieved smaller sizes on average when their silk was experimentally stolen in the laboratory (Edgerly et al., 2006), a shift in focus to examine offspring success as related to gregarious tendencies seems warranted. (4) Despite contrary evidence from laboratory experiments (Edgerly et al., 2006), females that join others in the field might benefit because they can share in the potentially costly effort to maintain the silk structure, as has been seen in colonial spiders (Uetz and Hieber, 1997). Sharing silk and spinning with others might allow webspinners to lay more eggs.

Potential Interactions: Food Resources, Predation and Dispersal

A 5-month (October through February) field experiment at Simla designed to test the important question of how food resources influence group success and dispersal failed because of persistent predation. Therefore, competition between individuals in colonies of various sizes and how that influences dispersal probability remains untested. The hypothesis was that dispersal by individuals can be triggered by low availability of food resources. If this were true, individuals in colonies established on boles with abundant food (epiphytic algae and lichens on the bark) would remain in place while those with limited food would disperse in search of food. The experimental design also integrated a test of predation as a trigger for dispersal; the high incidence of loss of individuals co-occurring with torn silk in natural colonies around the field station suggested such a connection. During the experiment, adult females placed on uncaged boles were not able to colonize because of high rates of predation. Dozens of attempts failed. Naturally dispersing adult females also showed up on the experimental boles, but they too were attacked. Even within a large exclusion cage designed to exclude predators, ants ultimately found entrances and predation could not be avoided and remained high. Failure of the experiment due to predation supports the hypothesis that predation is a major selective factor for *A. urichi.* Unfortunately, the roles of competition for food and predation in regulating dispersal and colony structure remain unknown.

How Do Embiopterans Compare to Other Arthropods That Rely on Silk for Protection?

For arthropods, silk as a domicile might be costly to produce and/or functions better when voluminous and thick. For insects, silk is mostly composed of simple amino acid building blocks such as glycine, alanine, and serine. This is true for embiopterans as well (Okada et al., 2008; Collin et al., 2009, 2011; Harper et al., 2021). Craig (1997) proposed that such silk proteins are not expensive for an insect to produce. However, the step energy (*sensu* Peakall and Witt, 1976) required to spin it might incur costs. Therefore, gregariousness by silk spinning insects might have evolved in a variety of lineages, to reduce such costs for immatures and/or for adults. Evidence from *A. urichi*, as reported above (based on Edgerly, 1988; Edgerly et al., 2006), suggests that nymphs might be more impacted than adults by the costs of spinning. Time budget analysis has shown that investment in silk production and spinning by *A. urichi* mothers increases after her eggs hatch (Edgerly, 1987b, 1988). Joining another adult female could be one mechanism helping her offspring gain support from another's silk if she were killed early in their development. In contrast to these examples, consider that while caddisflies incur a cost when silk is stolen from them and replacement is required, leaf-rolling gelechiid caterpillars surprisingly fared better when their silk was experimentally taken (Loeffler, 1996). Leoffler suggested that the displaced caterpillars moved onto fresh leaves and therein gained a benefit from better nutrition despite the increased investment in replacement webbing. That finding is interesting in that the natural situation is for larvae to stay within the silk domicile, safe from predation, despite declining food value.

Caterpillars often form groups that share the work of spinning their shelters as described in a review by Costa and Pierce (1997).

The authors reported that sociality is widespread within at least 20 families within the order Lepidoptera (Costa and Pierce, 1997, see Table 20.1). Some species are so-called patch-restricted while others are central-place foragers. The former encloses leaves (their food) with silk, which is depleted over time in the same manner as resources are exploited by colonies of embiopterans. Central-place foragers build a shelter and move out periodically to find fresh food and return to their tent to digest, rest and hide. These caterpillars display sophisticated signals, such as trail-marking and recruitment to high quality leaves (Fitzgerald and Peterson, 1983; Fitzgerald and Costa, 1986). The life history characteristics that shape caterpillar social behaviors are strongly influenced by foraging constraints and indeed, the heuristic model presented by Costa and Pierce (1997; see Figure 20-3) emphasized diversity of foraging styles. The three-dimensional tents serve as communication centers for the larvae as they tackle their often difficult and ephemeral food. These traits do not appear to typify webspinners beyond the silk-sharing tendencies. The communication signals we discovered are short-distance messages between egg-guarding females and possible intruders; not signals for an entire diffuse colony of silk-sharers (Dejan et al., 2013). Furthermore, based on my experience and that of Ross (2000b), webspinners from throughout their range can be reared in the laboratory on the same foods: romaine lettuce and lichens. This generalist diet is not true of caterpillars which often are highly specialized foragers. Costa and Pierce's review did not focus on the relationship of silk to potential predation or parasitism and dispersal risks, the factors I propose as having shaped the group-living behavior of *A. urichi.*

Costa and Pierce (1997) noted that gregariousness for tent-building caterpillars can evolve in an ecological context where family structure and relatedness are irrelevant. The authors discovered in their review of the literature that no one factor underlies the evolution of group-living for social caterpillars. Super colonies can form when sibling groups merge with others to share silk tents, and where relatedness is quantifiably low, hence the colonies are effectively non-kin groups (Costa and Ross, 2003). Promoting this assemblage, adult females preferentially lay their egg masses next to other egg masses. The offspring spin tents as sib-groups but as they develop, they merge with other groups and form super colonies. The relatedness coefficient for such a colony defined by who shares a tent shifts from 0.5 for young instar larvae to 0.25 for older larvae because of mixing later on (Costa and Ross, 1993).

Costa (2006) produced a marvelously insightful and helpful 767-page tome called "The Other Insect Societies." Digging into his thoughts about the evolution of social groups, I discovered that webspinners display a selection of his proposed common denominators of group formation; not unexpectedly given that the order Embioptera is featured as one of the chapters. These common denominators are biotic and abiotic ecological pressures, such as predation, parasitism, and environmental challenges. Costa also named thermal challenges as another pressure. I explored the question of thermal challenges for the tropical rainforest *A. urichi* and two Australian species that live in the hot environment of Magnetic Island in Queensland (Edgerly and Rooks, 2004). Silk domiciles provided little protection against strong solar radiation for the Australian species; instead, they displayed adaptive microhabitat selection to avoid overheating in the field. In our laboratory experiments, we found that effective heat shock proteins, not related to the silk's ability or lack thereof, prevented death due to heat shock for the Australian species (Edgerly et al., 2005). *Antipaluria urichi,* in contrast, suffered heat shock and death at comparably lower temperatures, not surprisingly given that the temperature range is narrow and moderate in their environment. My unquantified observations in the field suggested that rainfall, not heat, is an ecological challenge for the tropical rainforest *A. urichi* that can be solved by their silk covering (Stokes et al., 2018). Their silk functions as a raincoat, protecting the occupants from tropical downpours which would otherwise knock individuals from their bark perches and soak them—a problem that must be avoided given that they die very quickly if wet (personal observations). The question remains whether sharing silk with other individuals enhances the raincoat, which would also function underground where flooding would likely kill the detritivores like *Haploembia solieri, Oligotoma nigra,* and *M. incompta*. Costa (2006) also proposed that the common denominators for group-living promote communal oviposition, merging of unrelated groups and the absence of kin discrimination. These features hold true for *A. urichi* and a few other species for whom field censuses revealed similar facultatively colonial structure of their populations (see reviews Edgerly, 1997; Costa, 2006).

Dependence on silk for protection typifies other arthropods, such as species of Psocoptera and Acari. Some psocids live in enormous colonies, producing a thin silk mesh that can cover entire tree trunks (New, 1985). Little is known, however, about which advantages accrue to individuals in these colonies (reviewed in Costa, 2006). Mites in the genus *Schizotetranychus* also live in large groups within silk abodes (Saito, 1997). Differences between the mites and webspinners are great, however, making it hard to find common denominators, *sensu* Costa (2006). The mites are haplodiploid while webspinners are diploid, except for a few polyploids described by Stefani (1953, 1956) and Kelly et al. (2018). The spider mites cooperate in nest building, whereas for *A. urichi* at least, silk spinning is by individuals that merely join each other's silk, adding their own to the expanding 2-dimensional cover. The mites also cooperate in brood defense and show overlap of generations. Finally, Saito (1997) concluded in her review that the mite's complex sociality evolved in the context of kin selection. For other arachnids such as social spiders, kin selection plays a role in facilitating group formation (Avilés, 1997).

CONCLUSION

Opportunities for Future Studies on the Little-Known Embioptera

One webspinner species (*E. ramburi*) constructs a nest-like domicile where food is brought by the mother from the outside (LeDoux, 1958). This central-place forager exhibits a behavior seen in other insects that display more complex communication and social behaviors (reviewed in Costa, 2006).

Other webspinners are asexual; their behavior varies in unpredictable ways if one assumes that closely related individuals in a family will tend to be gregarious and gain advantages from sharing silk. For example, adult females of the Mediterranean species *H. tarsalis* (**Figure 2D**; formerly known as an asexual race of *H. solieri*, see Stefani, 1956) are aggressive; they fight, repel each other, and do not form groups (Kelly et al., 2018). In contrast, a closely related sexual species, *H. solieri* forms large aggregations in the same habitat (based on personal observation) and are gregarious in the lab (Kelly et al., 2018). Both species live in leaf litter and in underground burrows in the same habitat. The reasons for the differences in social tendencies for these two species are not known and are worth exploring. In contrast to *H. tarsalis*, the parthenogenetic *Rhagadochir virga* (formerly *Scelembia virga;* Ross, 1961) of Zambia forms large, apparently coordinated groups (personal observation). In laboratory cultures at Santa Clara University, adult females would periodically assemble on the sides of the container as if ready to disperse *en masse*. They also scattered their eggs in small clumps, did not stand guard but provisioned them with gathered pieces of lichens that perhaps would feed the neonates. The colony also had a lovely odor, like maple syrup. Was this a pheromone coordinating group behaviors? No field studies exist to date for this interesting gregarious species living in a seasonally dry-wet environment.

Predation-dilution effects, feeding facilitation and features of the environment can all play a role. For Embioptera, such a comprehensive review cannot be assembled because of scant information. On a positive note, more scrutiny is now possible as we have a phylogeny (Miller et al., 2012) to assist in making independent contrast tests of hypotheses focused on the evolution of group-living and difference in degree of reliance on silk. Environmental variation is known as well: based on my own experience, I have found species living in arid conditions (Australia), very wet and cool places (Andes of Ecuador), seasonally dry and seasonally wet (Zambia; Thailand), and places that are hospitable all year (tropical rain forests in Ecuador, Trinidad). For a comprehensive review of habitats and habits, see Ross (2000b)—an entomologist responsible for collecting from around the world most of the specimens now held in the collection at the California Academy of Sciences, United States. Some species experience egg parasitism and others do not. Some species suffer from nymphal parasites (Family Sclerogibbidae, Order Hymenoptera) and others do not (Ross, 2000b). Variation in environmental pressures is present and interestingly, as discussed above, except for size and color, morphological variation is not. Embioptera remain as one of the least studied groups of primitively social insects; the avenue is open for interesting research opportunities and further exploration of factors underlying variation in group-living awaits.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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Foraging Payoffs Change With Group Size in Kin and Non-kin Groups of an Argyrodinae Kleptoparasitic Spider, *Argyrodes miniaceus*

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Evolutionary transitions from solitary to group-living are ubiquitous in animal systems. While the fitness consequences of group size changes are often investigated, the long-standing debate on whether kinship is a prerequisite of sociality is still ongoing. In the current study, we used kleptoparasitic spiders *Argyrodes miniaceus* (subfamily Argyrodinae, Theridiidae) as a model system to assess the role of group size on the foraging payoffs of kin and non-kin groups. We set up laboratory-manipulated kin and non-kin foraging groups and used feeding occurrence and duration as proxies for foraging benefits and feeding latency and the number of host attacks as estimates of foraging costs. Compared to solitary individuals, feeding durations of successfully fed individuals in groups was not significantly different from that of solitary foragers in both kin and non-kin groups. The occurrences of feeding decreased significantly in group sizes two and above, in non-kin groups, and in group sizes three and above, in kin groups. In kin groups, groups size two had significantly shorter feeding latencies compared to other group sizes, even though feeding duration did not change systematically with group size. Similarly, the number of attacks from the hosts were highest in non-kin groups with more than two individuals and in kin groups with more than three individuals. The juxtaposition of kin and non-kin group showed that *A. miniaceus* enjoyed the highest foraging payoffs when being solitary or in small groups (group size two). However, host attacks appeared to hamper feeding occurrences in kin groups, which was not observed in non-kin groups. Our results contrast sharply with the feeding benefits of kinship recorded in kin-based groups of sub-social species present in related subfamilies in the Theridiidae.

Keywords: group-living, optimal group size, foraging payoff, kleptoparasitism, kin selection

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INTRODUCTION

The evolutionary pathways from solitary to sociality in a given taxonomic group have been investigated with a wide range of approaches, including theoretical (West et al., 2007), physiological (Oliveira et al., 2015; Kingwell et al., 2021), genetic (French, 2016; Warner et al., 2019; Yan and Liebig, 2021), and experimental (Schneider and Bilde, 2008; Gow et al., 2019; Abdi et al., 2020a,b).

During the early stages of sociality, i.e., when group-living and cooperation facultatively occurred in populations, it is clear that group size, a parameter related to social complexity (reviewed in Taborsky, 2021), could influence the fitness outcomes (Korb and Heinze, 2016; Brandell et al., 2021). While predictions of the theoretical models of group size effects on fitness have long been established (Giraldeau and Caraco, 2000), and the role of kinship (Giraldeau and Caraco, 1993; review of Platt and Bever, 2009) and other stochastic ecological effects, e.g., dispersal (Brown, 2016; Fernandez-Fournier and Avilés, 2018), have been discussed, it is important to apply these theories to the empirical animal systems. In group-living spiders, group size is an important determinant of fitness. Studies have reported that transitioning from solitary to certain group size would lead to broader dietary niches (Majer et al., 2018), weaker individual hunting ability (Harwood and Avilés, 2018), and the capture of larger prey (Guevara et al., 2011; Dumke et al., 2018). However, the importance of kinship in foraging has rarely been tested in group-living spiders (but see Auletta and Rayor, 2011; Yip and Rayor, 2013). Here, we used a group-living kleptoparasitic spider to test the foraging payoffs in different group sizes and to study the foraging outcomes in kin and non-kin groups depending on group size.

Kleptoparasitism refers to the behavior when an individual steals resources from other individuals (the same or different species). Kleptoparasitism reduces the amount of energy expended on foraging and has been reported in insects, spiders, birds, and mammals (reviewed in Iyengar, 2008). In the spider subfamily of Argyrodinae (Theridiidae), fewer than 20 of the named species (thus about 10% of the species) are group-living kleptoparasites (Whitehouse, 2011; Su and Smith, 2014). The group-living *Argyrodes* spiders use a variety of strategies to exploit the resources of their host. For the most part, these species live on the webs of their hosts (Su and Smith, 2014) or build a support web connected to the host's web, as in the case of *A. antipodiana* (Whitehouse, 1986). These kleptoparasitic animals feed on prey captured by the hosts, as well as the silk of the host web. In a few instances, *Argyrodes* species have been shown to prey on their hosts or the host spiderlings (Silveira and Japyassú, 2012). Nonetheless, specific foraging strategies depend on ecological conditions and vary among species (Whitehouse, 2011). Some group-living *Argyrodes* species use a "creep-up-and-share" strategy, in which they approach the food that a host is feeding and consume prey partially digested by the host, thereby eliminating the need to produce digestive enzymes by themselves (Whitehouse, 1997; Whitehouse and Lubin, 2005). This feeding strategy presumably maximizes food resource intake in terms of quantity and quality across all feeding tactics (Whitehouse, 1997) and could thus potentially be used to quantify food intake. However, the role of group size in their foraging payoffs has not been tested. Some researchers have considered the tolerance of *Argyrodes* individuals when conducting "creep-up-and-share" feeding behavior as a form of cooperative foraging (Whitehouse, 2011). The Argyrodinae is a subfamily in the Theridiidae, which contains a number of sub-social species (e.g., genus *Anelosimus*) (Agnarsson, 2004) where sociality could have been driven by kin selection (Su and Smith, 2014; Liu et al., 2016). Kleptoparasitic behavior undoubtedly brings resource-driven foraging dynamics into play in the evolution of sociality (Su et al., 2021). In nature, *Argyrodes* has been shown to form kleptoparasitic groups with kin as well as non-kin individuals (Su et al., 2018); these species therefore provide excellent opportunities to simultaneously test the role of group size and the contribution of kinship in the foraging payoffs during the early-staged evolution of sociality.

Cooperative behaviors ranging from reciprocal mutualism in non-kin groups to caste-differentiated eusocial colonies (Wilson, 1975). However, the evolutionary processes leading to the transitioning from solitary to sociality remain at the center of ongoing debates (Nowak et al., 2010, 2017; Abbot et al., 2011; Herre and Wcislo, 2011; Birch, 2017). Most researchers are in agreement that cooperative behavior has produced a diversity of biological innovations; however, the issue of whether kinship is a necessary evolutionary driver of the development and maintenance of an early-staged cooperation has yet to be conclusively determined. Furthermore, researchers have yet to conclude whether inclusive fitness theory (Hamilton, 1964) is applicable to general cases of sociality or whether it is simply a special instance of natural selection (Nowak et al., 2010, 2017). Researchers have developed several theories to address this controversy (e.g., Garcia and De Monte, 2013; Liao et al., 2015; Nowak et al., 2017); however, there is a lack of empirical evidence testing the role of kinship in early-stage evolution of sociality in nature.

Our focal system, the group-living *Argyrodes miniaceus* and its host *Nephila pilipes* is an ideal natural system to study the evolution of cooperative behavior and test hypotheses pertaining to the role of kinship in an early evolutionary form of cooperative behavior and sociality (Whitehouse, 2011). In the current study, we sought to determine whether foraging payoffs differ across members of different group sizes. Specifically, we measured feeding duration, feeding latency, and attacks from hosts as three variables to quantify foraging payoffs. We predicted that, if the group-living behavior in *A. miniaceus* were of a cooperative nature, the highest foraging payoffs of this form of kleptoparasitism would occur at group size larger than one. Secondly, we compared the trends of foraging outcomes in kin and non-kin groups. We predicted that if kin selection played a role in cooperative foraging in *A. miniaceus*, then the optimal size of foraging groups would be greater for kin groups than for non-kin groups. Absent of the latter pattern would suggest that kin selection might not be important in the evolution of group living in these spiders.

MATERIALS AND METHODS

Study Species

Argyrodes miniaceus (Doleschall, 1857) is a group-living kleptoparasitic spider species specialized in the invasion and exploitation of the webs created by female orb-weaving hosts

(Su et al., 2018), such as *Nephila* sp., including *Nephila pilipes* and *Trichonephila clavata* in Taiwan (personal observations of Yu in Namaxia, Kaohsiung, Taiwan). *Nephila pilipes* build two-dimensional orb webs where *A. miniaceus* forage, mate, and reproduce (personal observations of Yu); the orb web is made of frame threads as the basic structure, radial threads stretching from the central area of orb web (termed hub) to frame threads, and spiral threads connecting between radial threads (Wijerathna, 2016). In general, *N. pilipes* stay at hub to wait for prey, while *A. miniaceus* stay around the capture area composed of radial threads and spiral threads. Female *A. miniaceus* produce individual egg sacs in nearby vegetation or branches close to the host web. Male and female *A. miniaceus* both undergo four instars prior to maturation. In the field, it is common to find overlapping generations of *A. miniaceus* living in a web, i.e., adults, sub-adults, and juveniles of both sexes (first to third instars, Su et al., 2018) and the average group size is 4.8 ± 8.2 (Su et al., 2021). *Argyrodes miniaceus* utilizes four techniques when foraging on host webs: silk consumption, catching small insects, stealing wrapped food bundles from the host, and feeding with the hosts at the same time by creeping up to the food bundles (termed "creep-up-and-share"). Creep-up-and-share is their primary tactic among juvenile and adult *A. miniaceus* (usually two to three participants when conducting this feeding tactic, Su et al., 2018), indicating tolerance toward conspecifics during feeding; however, intraspecific aggression can still manifest in foraging as well as mating contexts. This unique form of group-living kleptoparasitism enables manipulation of kinship among group members in a laboratory setting.

Sample Collection and Rearing

We collected *A. miniaceus* (Araneae: Theridiidae) (referred as *Argyrodes* hereafter) and their hosts, female *Nephila pilipes* (Araneae: Nephilidae) (referred as *Nephila* hereafter), in Namaxia District of Kaohsiung in Taiwan (N 23° 16′ 17.9″, E 120° 43′ 33.3″), in which both species are commonly found. Juvenile and adult *Argyrodes* have been shown to share food with conspecifics; however, we focused exclusively on adult females for our experiments, due to the fact that foraging behavior is less observable in males. Adult female *Argyrodes* obtained from multiple host webs (i.e., minimal probability of sharing kinship) were used to assemble non-kin groups (Su et al., 2018). Kin groups were assembled by collecting egg sacs of *Argyrodes* in the field as well as those of spiders that had mated in the lab. Hatching involved attaching egg sacs to the side of 50 ml centrifuge tubes, at the bottom of which was placed moist cotton with a few drops of potable water for humidity. The tube opening was blocked using dry cotton to maintain suitable ventilation. Each egg sac contained 30–100 eggs, which hatched at room temperature after roughly 3 weeks (personal observations of Yu). The spiderlings were held in individual containers to prevent social interactions. At intervals of 2–3 days, the spiderlings were fed crickets that were partially digested by *Nephila*. After the *Argyrodes* spiderlings reached the third or fourth instar, they were reared in the web of *Nephila* in cages individually (BugDorm-1: 30 cm × 30 cm × 30 cm, BugDorm-6M1020 and 6E1020: 103 cm × 103 cm × 204 cm, MegaView Science Co., Ltd.) until they reached adulthood.

Experimental Set-Up

Female *Nephila* hosts were held in cages (103 cm × 103 cm × 204 cm, BugDorm-6M1020 and BugDorm-6E1020, MegaView Science Co., Ltd.) with sufficient space to build orb-webs. We removed *Nephila* individuals that were unable to complete a web after 1 day. Non-kin and kin groups of *Argyrodes* were assembled into groups of one to five individuals. Prior to initiating the experiments, *Argyrodes* were starved for at least for 48 h prior to be used in experiments but were fed *ad libitum* otherwise. For the sake of convenience in observation, individual *Argyrodes* were anesthetized using CO_2 and then marked with paint splotches of various colors (Sharpe and Avilés, 2016; Luminous Powder Kit #1162A, BioQuip Products, Inc.). Experiment groups were assigned to cages randomly.

Behavioral Experiment

Experiments on non-kin groups were conducted from January 2019 to November 2020. Experiments on kin groups were conducted in March, April, and July of 2021. All experiments were conducted during the day. Each experimental session involved placing a live cricket (*Gryllus bimaculatus*) weighing ∼ 0.3 g (Robinson and Robinson, 1973) within the capture area of the *Nephila* host web. After capturing the prey, *Nephila* generally returned to the central area of the web (i.e., the hub) to manipulate the prey into food bundles. As soon as the *Nephila* began handling the prey, we started recording the feeding session using video cameras (Sony FDR-AX40 and HDR-PJ675, China). For each *Argyrodes*, we documented the feeding duration, feeding latency, and the number of attacks from *Nephila*, based on observations of the recordings. The variables were as follows:

1. Feeding duration: Feeding behavior of each *Argyrodes* was defined as the amount of time in which the mouthpart of *Argyrodes* was in direct contact with food bundles of *Nephila*. The length of feeding duration was used as a proxy for foraging benefit gained based on the "creep-up-and-share" strategy, wherein feeding duration using creep-up-and-share strategy is an important indicator of foraging benefit to gain body mass, and presumably contributes to later probalility of reproduction (see Whitehouse, 1997).

2. Feeding latency: Feeding latency was defined as the duration between the point at which *Nephila* began handling the prey and the point at which the mouthpart of each *Argyrodes* first came into contact with the prey. Feeding latency was used to estimate the foraging cost associated with the "creep-up-and-share" strategy. If a spider did not feed throughout the experiment, we assigned the latency a value of 20 min (the longest possible duration of the non-feeding category given the duration of the observations, see data analysis).

3. Number of host attacks: When *Nephila* detected the movements of *Argyrodes*, they either vibrated the web with their legs in a menacing manner (referred to as *vibration* hereafter) to expel *Argyrodes* away or captured *Argyrodes* directly (referred to as *capture* hereafter). We recorded the occurrences of *vibration* and *capture* as the number of attacks throughout the feeding

session as another aspect of foraging cost associated with the "creep-up-and-share" strategy.

Data Analysis

All statistical analyses were performed in R (v 4.0.2; R Core Team, 2020). Prior to any analyses, we square-root transformed feeding duration and cube-root transformed feeding latency. We further transformed raw feeding latency as the absolute difference from the maximum value (20 min). The transformed feeding latencies were zero-inflated but otherwise continuous, a structure best described by the Tweedie distribution (Tweedie, 1984). After the transformation, a value of 0 denoted maximum latency (i.e., an *Argyrodes* did not feed), and smaller values corresponded to longer raw latency. Data for feeding duration shared the same feature. We therefore used generalized linear models (GLMs) with Tweedie distributions (*glmmTMB* package; Magnusson et al., 2021) to perform subsequent analyses. We constructed separate linear models for feeding duration and feeding latency in non-kin and kin groups, respectively. In each linear model, we began by including group size, initial distance of each *Argyrodes* from the hub, the sum of vibrations and captures from the host (hereafter "attacks"), and ambient temperature as predictors. We did not detect significant collinearity among predictors based on visual inspection of data and variance inflation factor values (**Supplementary Figure 1** and **Supplementary Table 1**; Zuur et al., 2008). We eliminated each predictor in turn and used Akaike Information Criteria (AIC) score difference from the full model ($\leq$2) to evaluate the contribution of each predictor. We also tested models with two-way interactions, but those models were inferior in all cases (Model 6, **Supplementary Tables 2**, **3**). Using GLMs with the Tweedie distribution, we constructed and tested two models: a zero-inflated model and a conditional model. In the zero-inflated model, the GLM examined the effect of each predictor in causing more zeros in the response variable. In the conditional model, the GLM tested the relationship of each predictor with non-zero values of the response variable, equivalent to a regular GLM.

When testing the effect of group size on vibrations and captures from the host, we employed generalized linear mixed models (GLMMs) with suitable distributions (Poisson, generalized Poisson, or negative binomial (**Supplementary Tables 4A,C**, **5A,C**) using package *lme4* and *glmmTMB* (Bates et al., 2015; Magnusson et al., 2021). In each GLMM, we included group size as a fixed variable and the identity of the *Nephila* host (host ID) as a random variable. We did not individually mark *Nephila* hosts in the earlier experiments, so we were unable to keep track of host identity in those experiments (**Supplementary Table 7**). Therefore, to gauge the influence of missing host IDs on statistical outcomes, we performed statistical analyses considering the identity of unidentifiable hosts in two extreme scenarios: (1) all unidentifiable hosts were assumed to be a sample of identified hosts. Under this scenario, we randomly assigned host IDs to unidentifiable *Nephila* hosts 100 times. (2) all unidentifiable hosts represented different individuals that did not overlap with any identified hosts. If the 101 models from the two scenarios were qualitatively similar in statistical outcomes, we would conclude that the influence of missing host ID did not affect the results.

RESULTS

Feeding Duration

For non-kin groups, the optimal model included all predictors except attacks from hosts (Model 5, **Supplementary Table 2**). Longer distance to the hub, lower temperatures, and a group size larger than one (with the exception of group size of four) were all significantly associated with zero feeding duration (**Table 1A**). However, none of the predictors explained the length of non-zero feeding duration in non-kin groups (**Figure 1A** and **Table 1B**).

For kin groups, the full model and the model excluding distance to the hub were equally optimal, thus we report results from the full model here (Model 1, **Supplementary Table 2**). Similar to non-kin groups, longer distance to the hub, lower temperature, and a group size larger than two led to more zero feeding duration, even though the effect of distance was not significant (p = 0.065, **Table 1C**). The confidence interval of the parameter for distance suggested the non-significance was likely the result of a smaller effect, rather than an uncertainty of parameter estimation (**Table 1C**). Fewer attacks from the host were also associated with more zero feeding duration. This result in kin groups reflected the fact that attacks resulted from foraging activities of *Argyrodes* (i.e., non-zero feeding duration), during which they were in closer proximity to the host and incurred more attacks. Once the *Argyrodes* began feeding, more attacks from the host resulted in shorter feeding duration (**Table 1D**). Feeding duration of group sizes two to five was not significantly different to that of group size one in both kin and non-kin groups (**Figure 1B** and **Table 1D**).

Feeding Latency

For non-kin groups, the model excluding attacks from the host was the optimal model (Model 5, **Supplementary Table 3**). As in feeding duration, longer distance, lower temperature, and a group size larger than one (with the exception of group size of four) were significantly associated with maximal latency (i.e., no feeding; **Table 2A**). Higher temperature was also significantly associated with shorter feeding latencies (**Table 2B**). Feeding latencies of group size two to five were not significantly different from that of group size one (**Figure 2A** and **Table 2B**).

For kin groups, the full model and the model without distance were equally optimal, thus we report results from the full model (Model 1, **Supplementary Table 3**). In this conditional model, group size of two had shorter feeding latencies comparing to group size one (**Figure 2B** and **Table 2C**), again, no significance (p = 0.064, **Table 2C**) of distance was more likely due to a smaller effect. Higher temperature caused shorter feeding latencies. Groups consisting of two *Argyrodes* kin also had significantly shorter feeding latencies compared to other group sizes (**Figure 2B** and **Table 2C**).

TABLE 1 | The statistical results of feeding duration in response to group size, kinship, distance to the hub, ambient temperature, and host attacks for kin and non-kin groups.

Effect	Intercept	Group size two	Group size three	Group size four	Group size five	Distance	Temperature	Attacks	Sigma	Tweedie. power
(A) Non-kin group: zero-inflation model of feeding duration — Model 5										
Estimate	6.734	4.003	2.772	1.711	3.232	0.083	−0.419	–	2.225	1.095
SE	3.075	1.380	1.210	1.244	1.210	0.030	0.121	–	–	–
Z-value	2.190	2.900	2.290	1.375	2.670	2.764	−3.460	–	–	–
p-value	0.029*	0.004**	0.022*	0.169	0.008**	0.006**	0.0005***	–	–	–
2.5% CI	0.707	1.297	0.400	−0.728	0.860	0.024	−0.656	–	1.218	1.008
97.5% CI	12.762	6.708	5.144	4.149	5.604	0.142	−0.182	–	4.063	1.573
(B) Non-kin group: conditional model of feeding duration (exclude zero-inflated data) — Model 5										
Estimate	3.279	0.155	−0.145	−0.031	−0.115	−0.007	0.002	–	–	–
SE	0.465	0.162	0.167	0.144	0.147	0.006	0.015	–	–	–
Z-value	7.053	0.957	−0.870	−0.217	−0.784	−1.159	0.151	–	–	–
p-value	1.75*E* − 12***	0.339	0.384	0.828	0.433	0.246	0.880	–	–	–
2.5% CI	2.368	−0.163	−0.471	−0.313	−0.404	−0.018	−0.027	–	–	–
97.5% CI	4.191	0.474	0.182	0.251	0.173	0.005	0.032	–	–	–
(C) Kin group: zero-inflation model of feeding duration — Model 1										
Estimate	2.032	1.321	2.574	4.402	3.782	0.035	−0.171	−0.146	2.733	1.050
SE	2.230	0.944	1.019	1.226	1.055	0.019	0.077	0.062	–	–
Z-value	0.911	1.400	2.527	3.592	3.584	1.846	−2.210	−2.348	–	–
p-value	0.362	0.161	0.011*	0.0003***	0.0003***	0.065	0.0271*	0.019*	–	–
2.5% CI	−2.340	−0.528	0.578	2.000	1.714	−0.002	−0.322	−0.268	2.110	1.004
97.5% CI	6.403	3.171	4.571	6.805	5.850	0.072	−0.019	−0.024	3.539	1.381
(D) Kin group: conditional model of feeding duration (exclude zero-inflated data) — Model 1										
Estimate	2.773	0.116	0.192	0.069	−0.054	−0.002	0.019	−0.037	–	–
SE	0.471	0.134	0.182	0.299	0.244	0.005	0.016	0.017	–	–
Z-value	5.893	0.863	1.057	0.232	−0.220	−0.466	1.216	−2.242	–	–
p-value	3.79e − 09***	0.388	0.290	0.816	0.826	0.641	0.224	0.025*	–	–
2.5% CI	1.851	−0.147	−0.164	−0.516	−0.532	−0.012	−0.012	−0.070	–	–
97.5% CI	3.695	0.379	0.549	0.655	0.424	0.007	0.049	−0.005	–	–

, **, and * denote the significance at the 0.05, 0.01, and 0.001 level.CI means confidence intervals.* ***(A)*** *and* ***(C)*** *are the results for feeding success under the zero-inflation model for non-kin and kin groups.Significant results indicate strong association of a predictor variable with the occurrence of zeros.* ***(B)*** *and* ***(D)*** *are the results under the conditional model when zeros are removed, thus including only successfully fed individuals.Significance levels indicate the effect of a predictor variable on feeding duration of individuals, given that individuals successfully fed.*

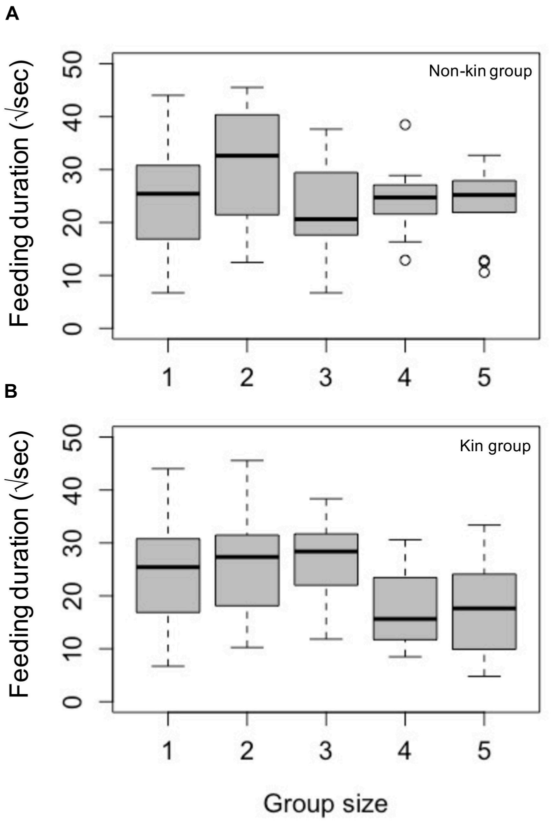

FIGURE 1 | Feeding duration (in seconds, square root transformed) as a foraging benefit in different group sizes of **(A)** non-kin and **(B)** kin laboratory assembled groups. Results shown that under the conditional model, where the zeros were removed, the feeding duration for the successfully fed *Argyrodes* showed nonsignificant difference among group sizes, including with the largest group size five. The data in non-kin groups showed a trend toward a peak in feeding duration in group size two, whereas kin groups do not have this trend. The samples sizes were: Group size one: $n = 12$; group size two: $n = 8$ for non-kin group and $n = 19$ for kin group; group size three: $n = 8$ for non-kin group and $n = 7$ for kin group; group size four: $n = 12$ for non-kin group and $n = 4$ for kin group; group size five: $n = 13$ for non-kin group and $n = 7$ for kin group.

Number of Host Attacks

In non-kin groups, the consensus from the 101 GLMMs showed that group sizes larger than two induced significantly more web vibrations from the host (the expelling behavior) than group size one (**Supplementary Table 4B**). Similarly, group sizes larger than three received significantly more web vibrations from the host in kin groups (**Supplementary Table 4D**). Group sizes did not have an effect on the number of captures by the host in both non-kin and kin groups (**Supplementary Tables 5B,D**). The results of the number of host attacks are summarized in **Figure 3**.

DISCUSSION

The model selection results indicated that group size consistently contributed substantially to the occurrence of feeding (i.e., the results of zero-inflation model), feeding duration, and feeding latency (i.e., the results under conditional model) in both kin or non-kin groups. Other factors, i.e., *Argyrodes* distance to the hub, ambient temperature, and attacks of hosts, were also important. Our results demonstrated that the occurrences of feeding in *Argyrodes* depended on group size in both kin and non-kin groups (**Tables 1A,C**) but host attacks only hampered feeding occurrences in kin groups. Compared to solitary feeding, the occurrences of feeding decreased in group size two to five in non-kin groups (except group size four) and group size three to five in kin groups (**Tables 1A,C**). Among individuals that got to feed, their feeding duration and latency were not significantly affected by group sizes, with the exception that *Argyrodes* in group size two of kin groups did not wait as long before commencing feeding (**Tables 1**, **2** and **Figure 2**). In addition to group size, *Argyrodes* feeding activities were hampered by lower ambient temperature and a longer distance from the hub. The risks of being chased away by *Nephila*, i.e., the number of vibrations, the primary technique of host attack, increased in group size three to five in non-kin groups and in group size four to five in kin groups (**Supplementary Tables 4B,D**). In general, our results showed an interesting pattern that the occurrences of feeding in *A. miniaceus* decreased in larger groups. Once an *Argyrodes* individual started feeding, feeding duration did not differ with respect to group size. Accordingly, *Argyrodes* enjoyed the highest foraging payoffs when solitary compared to any group size. The only notable exceptions were individuals in non-kin groups of size four and kin groups of size two, which had similar foraging payoffs to those of solitary individuals (**Tables 1A,C**).

We demonstrated experimentally that the per capita foraging payoffs of *Argyrodes* decreased with groups of any size compared to solitary individuals. Since resource size was fixed in our experiments, per capita foraging payoffs decreased when there were more foragers in a group. This result aligned with the field observations where the resource size was a determinant of group size in *Argyrodes* (Su et al., 2021). In natural populations, the average group size of *A. miniaceus* is 4.8 ± 8.2 (Su et al., 2021), yet we showed that individuals foraging in groups would suffer lower foraging payoffs (**Table 1D**) and more host attacks (**Supplementary Table 4D**). Therefore, the results of our experimental setup using the average size of food resource (0.3 g of food, Robinson and Robinson, 1973) did not correspond to the observed average group size in the natural populations. There are several possible mechanisms that would maintain an average group size of ∼5 individuals in the field. The first mechanism is the limited dispersal model, in which group-living results as a consequence of a potentially high cost of dispersal as in some social spiders (reviewed in Whitehouse and Lubin, 2005; Avilés and Guevara, 2017). For *Argyrodes* spiders, *Nephila* webs represent rare resource patches (Su et al., 2021). Spiders born on the same host web might therefore be forced to tolerate one another, even though foraging payoffs may be lower than when spiders are solitary on a web. Under this scenario, creep-up-and-share dynamic might evolve such that each member on the web takes turns distracting the host and allowing other members to feed more safely. The producer-scrounger model could be an alternative explanation for such group-living behavior in spiders

TABLE 2 | Statistical results of feeding latency in response to group size, kinship, distance to the hub, ambient temperature, and host attacks for kin and non-kin groups.

Effect	Intercept	Group size two	Group size three	Group size four	Group size five	Distance	Temperature	Attacks	Sigma	Tweedie. power
(A) Non-kin group: zero-inflation model of feeding latency — Model 5										
Estimate	6.734	4.002	2.771	1.710	3.231	0.083	−0.419	–	0.629	1.049
SE	3.074	1.379	1.209	1.243	1.209	0.030	0.121	–	–	–
Z-value	2.190	2.901	2.292	1.375	2.672	2.765	−3.461	–	–	–
p-value	0.028*	0.004**	0.022*	0.169	0.008**	0.006**	0.0005***	–	–	–
2.5% CI	0.709	1.298	0.401	−0.727	0.861	0.024	−0.656	–	0.371	1.003
97.5% CI	12.760	6.705	5.142	4.147	5.601	0.142	−0.182	–	1.067	1.496
(B) Non-kin group: conditional model of feeding latency (exclude zero-inflated data) — Model 5										
Estimate	0.979	0.108	0.130	0.035	0.177	−0.001	0.043	–	–	–
SE	0.367	0.130	0.126	0.115	0.115	0.004	0.012	–	–	–
Z-value	2.666	0.837	1.031	0.309	1.544	−0.128	3.629	–	–	–
p-value	0.008**	0.403	0.302	0.757	0.123	0.898	0.0003***	–	–	–
2.5% CI	0.259	−0.146	−0.117	−0.189	−0.048	−0.009	0.020	–	–	–
97.5% CI	1.699	0.362	0.377	0.260	0.401	0.008	0.066	–	–	–
(C) Kin group: conditional model of feeding latency (exclude zero-inflated data) — Model 1										
Estimate	0.816	0.246	−0.029	0.232	0.011	0.002	0.048	−0.013	–	–
SE	0.329	0.092	0.131	0.184	0.156	0.003	0.011	0.010	–	–
Z-value	2.482	2.670	−0.218	1.257	0.073	0.495	4.393	−1.273	–	–
p-value	0.013*	0.008**	0.828	0.209	0.942	0.621	1.12e-05***	0.203	–	–
2.5% CI	0.172	0.065	−0.286	−0.129	−0.295	−0.005	0.026	−0.032	–	–
97.5% CI	1.460	0.427	0.228	0.592	0.318	0.008	0.069	0.007	–	–

, **, and * denote the significance at the 0.05, 0.01, and 0.001 level.CI = confidence intervals of 2.5 and 97.5%.* ***(A)*** *represents the feeding latencies including individuals that did not feed during the feeding bouts (maximum value) under the zero-inflation model.The significant results indicate strong association of a predictor variable with zeros (feeding latency reaches maximum) and non-zeros (fed individuals).* ***(B)*** and ***(C)*** *are the results under the conditional model, which excludes "zeros," thus reflecting the significance of the various predictor variables on the waiting time of successfully fed individuals.*

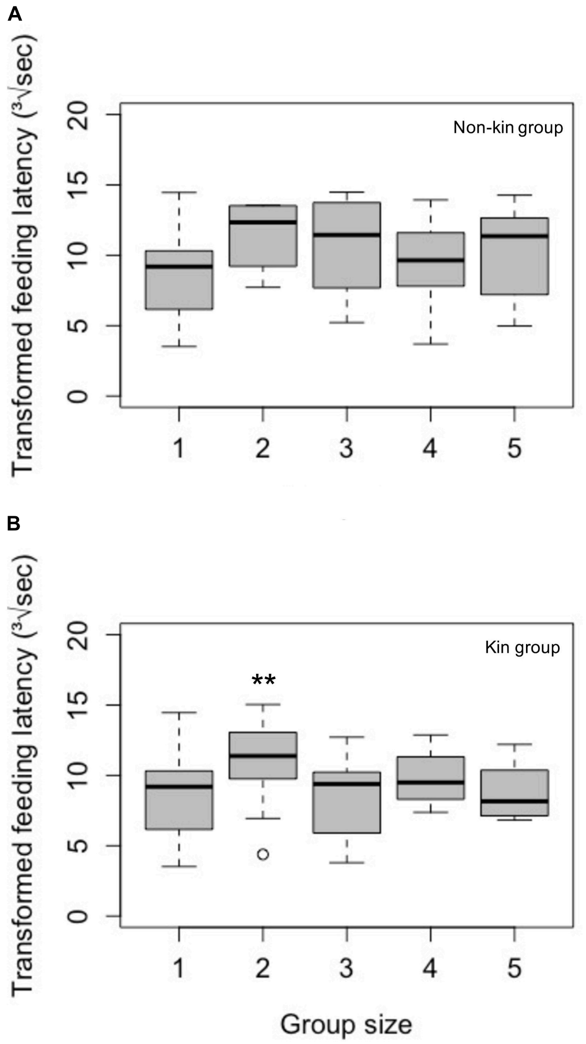

FIGURE 2 | Feeding latency as a foraging cost of **(A)** non-kin and **(B)** kin groups in different group sizes. Latencies in the figure are expressed as the absolute difference from the maximum latency (20 min) after taking the cube-root of the raw data). Values of zero on the y-axis denote a raw latency of 20 min; larger values of transformed latency denote shorter raw latency. Results shown that under the conditional model, where cases of no feeding during the observation period (maximum latency) were excluded. While most of the comparisons of feeding latency across group sizes were not significant, group size two in kin groups showed longer feeding latencies, thus higher costs, than other group sizes. Because the data were cube transformed and further transformed raw feeding latency as the absolute difference from the maximum value, larger values here represent shorter latency. The sample sizes were: Group size one: n = 12; group size two: n = 8 for non-kin group and n = 19 for kin group; group size three: n = 8 for non-kin group and n = 7 for kin group; group size four: n = 12 for non-kin group and n = 4 for kin group; group size five: n = 13 for non-kin group and n = 7 for kin group (exclude the data of transformed feeding latency = 0). ** means significant difference between group size one and two at $p < 0.01$.

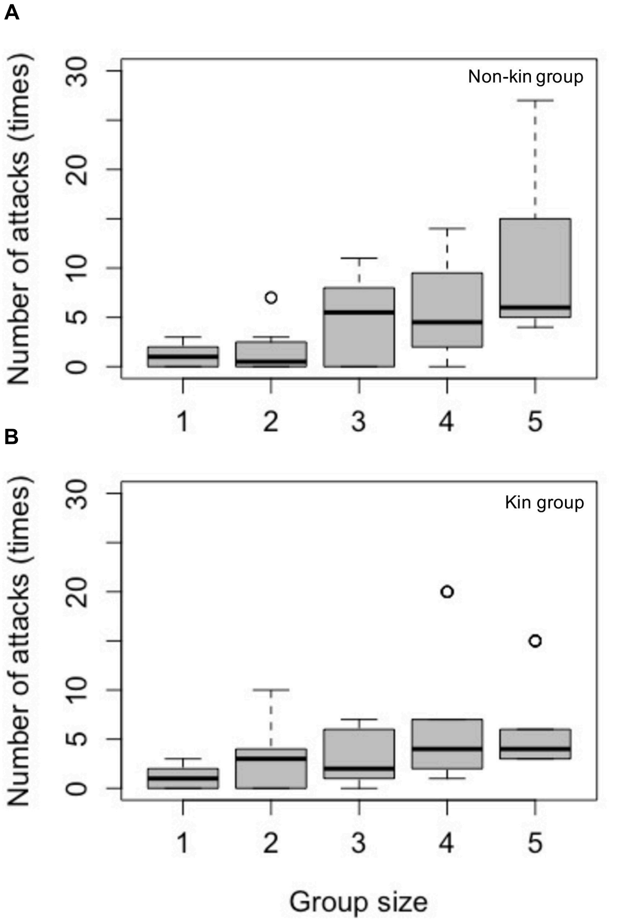

FIGURE 3 | Numbers of *Nephila* attacks (the sum of vibrations and captures) as a foraging cost of **(A)** non-kin and **(B)** kin groups in different group sizes. Two kinds of attacks are included in the analyses. The vibration, which is a tactic for *Nephila* to chase *Argyrodes* away, occurred more frequently. The number of this kind of attacks increased when group size > 2 in non-kin groups (see also **Supplementary Table 4B**) and when group size > 3 in kin groups (**Supplementary Table 4D**). The number of captures, a tactic to kill the *Argyrodes*, occurred in very low frequency and showed no difference across group sizes (see also **Supplementary Table 5**). Group size one: n = 14; group size two: n = 8 for non-kin group and n = 13 for kin group; group size three: n = 6 for non-kin group and n = 6 for kin group; group size four: n = 4 for non-kin group and n = 6 for kin group; group size five: n = 5 for non-kin group and n = 5 for kin group.

(i.e., crab spiders, Dumke et al., 2016). This model predicts a group-living outcome because each individual may have the opportunity of being the producer (i.e., the first *Argyrodes* to locate and feed on the trapped prey on the host's web), which enjoys higher feeding payoffs than the scroungers. In our experiments, spiders that fed first did have longer feeding duration both in kin and non-kin groups, even though the difference did not reach statistical significance (feeding duration in kin group: 25.59 vs. 21.35, t = 1.24, df = 26.07, p = 0.23; non-kin group 28.00 vs. 22.64, t = 1.95, df = 29.37, p = 0.06; **Supplementary Figure 2**). Further tests on dispersal costs, as well as the interactions between *Argyrodes* spiders and their hosts would help assess the validity of these hypotheses.

Group size in *Argyrodes* species tends to be positively correlated with food abundance (Cangialosi, 1990a,b; Agnarsson, 2003, 2011; Su et al., 2021). However, the contribution of kinship in promoting group-living has been inconclusive. Although living with kin can sometimes be beneficial (reviewed in Taborsky et al., 2021), the opposite has also been observed in a wide range of taxa (e.g., Zöttl et al., 2013; Dunn et al., 2014; Foster and Briffa, 2014; Thompson et al., 2017; Schweinfurth and Taborsky, 2018). In *Argyrodes* spiders, individuals sharing a web in the field could have higher relatedness than what would be expected by chance (Su et al., 2018), which could support the hypothesis that forming foraging groups with kin further increases fitness. Our results revealed that, regardless of kinship, groups of any size suffered higher foraging costs than solitary individuals, and that relatedness among members did not lead to larger optimal group size (**Figure 3** and **Supplementary Tables 4B,D**). *Argyrodes* spiders are belong to the same family to the sub-social Theridiidae spiders (Agnarsson, 2004), and it has been hypothesized that *Argyrodes* spiders may represent a very early stages of sub-sociality (Whitehouse, 2011). However, our findings suggested that kin selection hypotheses, the explanation for some social spiders (see Schneider and Bilde, 2008; Yip and Rayor, 2013; Ruch et al., 2014; review in Yip and Rayor, 2014), may not apply in our system.

We used feeding duration, feeding latency, and risk of being attacked by hosts to estimate the foraging payoffs of *Argyrodes*. Overall, our results indicated that group living may not provide much (if any) foraging benefit, and kinship did not affect group size-foraging payoff dynamics. From our field observation, if an adult female *A. miniaceus* fed successfully using creep-up-and-share tactic in a feeding trial, it could have enough reproductive energy to produce egg sacs (Yu personal observation). It would be necessary to conduct further work on other group-living Argyrodinae species to verify the appropriateness of using foraging payoffs as a fitness proxy. Moreover, due to the experimental design our non-kin and kin groups might also differ in aspects other than kinship (e.g., prior social experience), which prevented us from statistically examining the effect of kinship on group formation. However, the fact that group size negatively influenced foraging payoffs in both kin and non-kin groups was still noteworthy. Further experiments using individuals with the same social experiences and in similar development stages are required to fully test the role of kinship in facilitating or deterring group-living in these spiders. We also advocate *Argyrodes* spiders to be a tractable model system with which to test more hypotheses regarding the origin of sociality, e.g., sibling cooperation and parent-offspring competition, that are not often considered in empirical studies (see example of earwigs, Kramer et al., 2015 and review in Kramer and Meunier, 2019).

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.

AUTHOR CONTRIBUTIONS

C-NY conducted fieldwork, lab work, and wrote the methods and results. Y-CS designed and supervised the project, acquired the facility, and conducted the writing. C-YK designed and conducted the data analyses and conducted writing of the methods and results. H-CL assisted the analyses and edited manuscript. 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.2022.813777/full#supplementary-material

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