MULTIMODAL MATING SIGNALS: EVOLUTION, GENETICS AND PHYSIOLOGICAL BACKGROUND

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MULTIMODAL MATING SIGNALS: EVOLUTION, GENETICS AND PHYSIOLOGICAL BACKGROUND

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EDITORIAL
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Editorial: Multimodal Mating Signals: Evolution, Genetics and Physiological Background

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Editorial on the Research Topic

Multimodal Mating Signals: Evolution, Genetics and Physiological Background

When communicating vital information, such their attractiveness to potential mating partners or their unpalatability to predators, species are not restricted to signaling in a single mode. Instead they may produce signals in two or more modes, aka multimodal signaling. Multi-modal (pre)mating signals may be a combination of acoustic and visual signals, such as those used by birds (Ota et al., 2015; Cooney et al., 2018), frogs (Halfwerk et al.), and fish (de Jong et al.). In addition, combinations of acoustic and chemical signals are used by myrmecophilus butterflies in their complex parasitic interactions with ants (Casacci et al.), and many aposematic species use a combination of visual and chemical to ward off predators (Rojas et al.). Thus, multiple signals may interact with each other, and understanding the evolutionary pressures on these signals requires a thorough understanding of these interactions.

In general, signals can be under sexual and natural selection pressures. Rojas et al. discuss in detail how the complex interplay between natural and sexual selection can influence aposematic displays. Species with high within-population variability are particularly excellent models to determine how different selection forces affect the evolution of warning signals, but unfortunately there are not many studies focusing on intraspecific variation within and between populations that consider both natural and sexual selection (Rojas et al.). One exception is poison frogs, where the most well-defended males are also the most attractive ones (Maan and Cummings, 2008), so that natural and sexual selection work in concert. In invertebrates, such as beetles, *Heliconius* butterflies and day-flying moths, studies focus mostly on predator-imposed frequency-dependent selection on color variation. How variation in color interacts with chemical signals, such as those used as chemical defenses against predators, and sex pheromones, is now starting to be investigated (Gordon et al., 2015; Rojas et al., 2019).

The level and extent of natural and sexual selection pressures depends on the receivers of the signal. When under sexual selection, signals can be received by both competitors (intra-sexual communication signals) and by the choosing sex (inter-sexual communication signals). Vedenina and Shestakov studied the interplay between female preferences and male-male interactions in the cricket *Gryllus bimaculatus* to evaluate which sensory modalities are an indicator of male dominance status. As females mounted winners and losers equally often, the authors bring forward the interesting, and generally ignored, point that signals used by females may differ from signals used by males. Similarly, Rajaraman et al. show that both males and females in the bushcricket *Onomarchus uninotatus* may signal to each other, albeit with different types of signals and different forms of responses: males have acoustic calls and respond to vibrating females with vibrotaxis, while

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females use vibratory signals and respond to males with phonotaxis or tremulation, depending on the distance between the sexes.

Compared to unimodal signals, multimodal signals are special in the sense that they may improve associative learning. As there is more information per unit time, interactions may be more efficient than each signal by itself (Rojas et al.). Multimodal signals may also be backup signals: if one channel is blocked, a receiver can still receive the message through the other channel (Halfwerk et al.). A nice example of a redundant signal is given by Deodhar and Isvaran in the lizard *Psammophilus dorsalis*, where males use behavioral as well as color signals, which are strongly correlated. However, as different signal traits were affecting competitors, mates, and predators differentially, different signal components may be less redundant, but rather influenced by multiple selection pressures.

Multimodal signals may also be multiple messages which can convey different information at the same time, for example species identity as well as intention to mate (Halfwerk et al.). Importantly, multimodal signal evolution depends not only on who the receivers are, but also how signals are processed by the receiver's sensory systems. Halfwerk et al. review the current state of knowledge on how multimodal signals are integrated, ranging from humans and other vertebrates to insects. Multimodal integration is well-studied in humans, but also prevalent in birds, bees, fish, insects and frogs. Since interactive perception may give different results than each signal separately, Halfwerk et al. plea for an integrated approach to assess multimodal percepts.

Complicating factors that may affect the evolution of multimodal communication include learning, as well as interactions with the biotic and abiotic environment. Dion et al. extensively reviewed evidence that learning affects the evolution of (pre)mating signals in spiders and insects, meaning that sexual interactions are modified after experience. This effect is also nicely shown by Bunting and Hedrick, who found that previous encounters between males in the cricket *Gryllus integer* alters the songs of dominant (winning) males, but not subordinate (losing) males. As Dion et al. show, both short and long term memory of previous experiences impact lifetime mating behaviors in many insect and spider species, and both can learn multiple types of information. However, almost all studies have focused on visual or acoustic or olfactory signals, instead of possible combinations of interactions, so that it remains unclear whether and how learning of multimodal signals could be involved in the evolution of multimodal signaling.

Finally, biotic and abiotic environmental factors may also influence multimodal signal evolution. de Jong et al. determined the effect of anthropogenically induced noise on courtship behavior in the painted goby (*Pomatoschistus pictus*), in which visual and acoustic signals are used by males to attract females. As female gobies paid more attention to visual than acoustic signals in noisy environments, this study brings forth the important point that selection pressures on multimodal signals may differ depending on the level and extent of interfering habitat backgrounds. Background interference can take many forms, from anthropogenically produced noise to naturally-occurring heterospecific signals. The fact that social environment affects multimodal signals is best illustrated by the complex multimodal signaling in myrmecophilous butterflies, as reviewed by Casacci et al. These butterflies have developed amazing obligate-parasitic life history strategies with Myrmica ants to fulfill their life cycle, and use visual, chemical and acoustic signals to attract and deceive ant workers. Casacci et al. put forward the appealing hypothesis that the amazing signal complexity between these butterflies and ants may have boosted the evolution of specialized life cycles, thereby increasing butterfly diversity.

In conclusion, the contributions presented in this Research Topic highlight the wide variety of possible mechanisms underlying multimodal signaling and mate preferences for multiple traits, and give several ideas for further research in this framework. To fully understand the evolution of multimodal signaling it is essential to investigate the genetic basis of the different signals and how these different signals are processed by the receiver's sensory systems, as well as identifying the interacting selection pressures acting on multimodal signals.

AUTHOR CONTRIBUTIONS

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

REFERENCES

Cooney, C. R., MacGregor, H. E. A., Seddon, N., and Tobias, J. A. (2018). Multimodal signal evolution in birds: re-examining a standard proxy for sexual selection. *Proc. R. Soc. B Biol. Sci.* 285:20181557. doi: 10.1098/rspb.2018.1557

Gordon, S. P., Kokko, H., Rojas, B., Nokelainen, O., and Mappes, J. (2015). Colour polymorphism torn apart by opposing positive frequency-dependent selection, yet maintained in space. *J. Anim. Ecol.* 84, 1555–1564. doi: 10.1111/1365-2656.12416

Maan, M. E., and Cummings, M. E. (2008). Female preferences for aposematic signal components in a polymorphic poison frog. *Evolut. Int. J. Organ. Evol.* 62, 2334–2345. doi: 10.1111/j.1558-5646.2008.00454.x

Ota, N., Gahr, M., and Soma, M. (2015). Tap dancing birds: the multimodal mutual courtship display of males and females in a socially monogamous songbird. *Scient. Rep.* 5:16614. doi: 10.1038/srep16614

Rojas, B., Mappes, J., and Burdfield-Steel, E. (2019). Multiple modalities in insect warning displays have additive effects against wild avian predators. *Behav. Ecol. Sociobiol.* 73:37. doi: 10.1007/s00265-019-2643-6

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Why Do Males Use Multiple Signals? Insights From Measuring Wild Male Behavior Over Lifespans

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Why animals commonly use multiple conspicuous and presumably costly signals is poorly understood. Tests of evolutionary hypotheses comprehensively covering the signaling repertoire in wild populations are crucial to establish biological relevance, yet are relatively rare. We tested a key hypothesis for the maintenance of multiple signals in a wild population of the lizard, *Psammophilus dorsalis*, specifically whether multiple signals are maintained as multiple messages directed at different receivers. In addition, we also examined patterns in covariation of signals as an initial test of an alternative hypothesis, that multiple signals may be maintained as redundant signals; such traits are proposed to convey and reinforce the same component of information and are expected to be strongly correlated. Breeding male *P. dorsalis* display from prominent rock perches within their territories, which overlap multiple female home ranges in rocky open habitats. We repeatedly measured the display behavior, covering the entire signaling repertoire, of individually-tagged wild males on their territories over their lifespans. We quantified patterns of covariation in multiple traits and their relationship with multiple receiver contexts, specifically competitors, mates and predators. We also examined the association between male signaling and indices of lifetime fitness. Males commonly used multiple signals, including behavioral signals and a rare dynamic color signal. These traits were strongly correlated and seemed largely directed toward females, suggesting that they were primarily maintained as redundant signals through female choice. However, other selection pressures also appeared to be important. One color trait seemed to be directed at competitors, providing limited support to the multiple receiver hypothesis. Several traits were reduced in the presence of predators, suggesting that they carry the cost of increased predation risk. Thus, multiple selection pressures, primarily female choice and predation risk, appear to affect male signaling. Finally, signaling traits appeared to influence a measure of lifetime reproductive success, providing rare evidence for the biological relevance of signaling traits under natural contexts.

Keywords: communication, multiple signals, redundant signal, multiple message hypothesis, sexual selection, reptiles

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INTRODUCTION

Animals often employ a diverse range of conspicuous traits to signal to conspecifics and occasionally, to heterospecifics (Brodie, 1977; Bradbury and Vehrencamp, 1998; Rek and Magrath, 2016). Given the large costs of signaling (Halfwerk et al., 2014), why do animals use multiple signals rather than a single signal to advertise their quality (Johnstone, 1996)? A key set of hypotheses explaining the evolution and maintenance of multiple signals within a population proposes that multiple signals represent uncorrelated independent pieces of information (multiple message and multiple receiver hypotheses; Moller and Pomiankowski, 1993; Johnstone, 1996). According to the "multiple message hypothesis," multiple signals can evolve in a population if each signal conveys a different component of information about the overall quality of the signaler (Bókony et al., 2006; Bro-Jørgensen and Dabelsteen, 2008; Martín and López, 2009; Plasman et al., 2015). For example, in the Dickerson's collared lizard (*Crotaphytus dickersonae*), blue color of the skin appears to convey resource-holding potential while the blackness of the collar indicates immune condition (Plasman et al., 2015). In addition, multiple traits could be maintained if they are used in different contexts, or directed toward different receivers (Endler, 1992; Marchetti, 1998; Andersson et al., 2002; Loyau et al., 2005). In the wild, two common contexts in which individuals communicate are predation and mate-acquisition. Furthermore, within the mating context, individuals may use certain traits to signal to potential mates and others to signal to competitors. For example, a red carotenoid collar is reported to be involved in contest competition and an elongated tail in mate choice in the red-collared widowbird (*Euplectes ardens*) (Andersson et al., 2002). Such use of different traits might evolve either to avoid confusion regarding the intended receiver, and/or because different information may be communicated toward the different receivers. For example, individuals may convey information on their genetic quality to potential mates, their motivation to defend a territory/mate to potential competitors, and their ability to escape an attack to predators. Predation pressure can influence signal evolution, by favoring conspicuous displays directed specifically at the predator (Brodie, 1977; Caro, 1986) or by modifying the payoffs of signals functioning in other contexts, such as mate attraction (e.g., paler coloration in guppies from high-predation populations compared to those in low-predation populations; Endler, 1992).

While empirical support is arguably the greatest for the multiple-message hypothesis (Martín and López, 2009; Bro-Jørgensen, 2010; Plasman et al., 2015), alternative hypotheses have also been proposed for the maintenance of multiple signals. Several of these propose that multiple signals represent redundant pieces of information and are correlated (Moller and Pomiankowski, 1993; Candolin, 2003; Hebets and Papaj, 2005; Bro-Jørgensen, 2010). According to the "redundant signal" or "back-up signal" hypothesis, multiple signals convey, and reinforce the same component of information about the signaler's quality (Moller and Pomiankowski, 1993; Johnstone, 1996). For example, in the blue tit (*Cyanistes caeruleus*), two visual signals and an acoustic signal all appear to indicate the level of genetic diversity in a male (Ferrer et al., 2015). The probability of making a wrong decision and time taken to make a decision are lower if multiple traits, rather than a single trait, are evaluated (Smith and Evans, 2008). Red junglefowl (*Gallus gallus*) hens react faster to a rooster's food-alerting signal, if the hens are simultaneously exposed to rhythmic head-movements as well as vocalizations of the rooster (Smith and Evans, 2008). Alternatively, redundant signals may consist of an informative high-cost signal accompanied by less informative low-cost signals that improve the detectability and/or discriminability of the high-cost signal (Rowe, 1999). It is also possible that multiple mechanisms (e.g., both multiple message and redundancy in information) are simultaneously involved in the maintenance of multiple signals (Bro-Jørgensen and Dabelsteen, 2008).

An important aspect of examining these hypotheses for the maintenance of multiple traits is to examine the biological relevance of these traits, i.e., their relative contributions to fitness. Relationships of individual or a few traits with measures of fitness have been reported across a wide array of taxa [e.g., frillneck lizards (Hamilton et al., 2013), wolf spiders (Rundus et al., 2011), collared flycatcher (Qvarnstrom, 1997)]. However, where multiple signaling traits occur, the relationship between individual traits and the signaler's fitness may be complex (Candolin, 2003; Roberts et al., 2006). For example, the mate-attraction success of male ornate tree lizards (*Urosaurus ornatus*) could be explained only when male display-traits were considered in a multivariate rather than an individual trait analysis (Hamilton and Sullivan, 2005). Therefore, it is important to measure the entire signaling repertoire (Rek and Magrath, 2016), decipher the relationships among individual traits, and quantify their relative contributions to fitness. Furthermore, since behavioral signals are inherently variable, multiple measurements of signaling behavior, preferably distributed over an individual's lifetime, are needed to characterize well the level of signaling that the individual engages in.

In addition, much of our understanding of the ecology and evolution of signaling traits is based on work carried out in captive or semi-captive conditions (but see Baird, 2013). However, unlike in these controlled conditions, where individuals are typically exposed to a limited selection regime, individuals in wild populations experience diverse selection pressures. While there is considerable understanding of how traits evolve under a given selection pressure (such as sexual selection, predation), and under specific contexts (Zuk et al., 1992; Hamilton et al., 2013), information on how multiple selection pressures act simultaneously on signaling traits is scarce.

We studied the maintenance of multiple signaling traits in a wild population of *Psammophilus dorsalis* by investigating the relative importance of different selection pressures on these traits under natural ecological and social contexts, and the relationship of these traits with measures of lifetime fitness. *P. dorsalis* males are known to use visual signals—complex body postures and movements—for intraspecific communication (Radder et al., 2006). There is no evidence for olfactory or acoustic communication in this species, allowing us to study the entire signaling repertoire in this species. We investigated visual signaling in males in relation to (a) female mate choice, (b) male-male competition, and (c) predation risk. To understand the functions of these signals, we examined their associations with different contexts (mates, competitors, predators). These relationships allowed us to assess whether multiple signals may be maintained as multiple messages directed at different receivers. A stronger relationship of some signals with mates and others with competitors and/or predators would provide support for the multiple receiver hypothesis. As an initial evaluation of redundant signal hypotheses, we also examined the correlations amongst the multiple signals. A strongly correlated set of signals associated with a single context would indicate that multiple male signals are redundant. Such covariation in signals is not expected under the multiple receiver hypothesis (Candolin, 2003; Hebets and Papaj, 2005) since the presence of different receivers in the vicinity of the signaler is unlikely to be correlated. Finally, to evaluate the biological relevance of multiple signaling traits, we examined their relationship with measures of male lifetime fitness.

METHODS

Study System

Psammophilus dorsalis is a diurnal, rock-dwelling, sexually dimorphic agamid lizard. Males are larger than females and display bright coloration during the breeding season (Deodhar and Isvaran, 2017), from May to September. Found exclusively on large flat rocks (henceforth sheet rocks), they perch on rocks and signal to conspecifics using body postures, movements and colors, and also reportedly react to heterospecifics (Radder et al., 2006). These lizards breed predominantly only during one breeding season (Deodhar and Isvaran, 2017). We performed this study in Rishi Valley, Andhra Pradesh, India (13° 32′N, 78° 28′E), from May 2011 to September 2013. The area experiences stark seasonality in temperature and precipitation (Deodhar and Isvaran, 2017) and primarily consists of thorny scrub vegetation and hilly terrain. At our study site, several predators such as common Indian monitor lizard (*Varanus* sp.), Indian fox (*Vulpes bengalensis*) and various species of snakes and birds of prey, have been observed to prey upon and interact with *P. dorsalis* (SD, personal observations).

Individual Identification

Adult males were tagged before the onset of the breeding season. Subsequently arriving adults and recruits were tagged as soon as possible. Lizards were captured by noosing and uniquely tagged using color-coded combinations of 4 ceramic beads. Beads were attached on the dorsal surface at the base of the lizard's tail using a procedure specifically developed for tagging lizards (Fisher and Muth, 1989). Body size (snout vent length) was measured using Vernier calipers (Mitutoyo) to the nearest millimeter. Handling time lasted a maximum of 15 min per individual. Lizards were released back at their capture-location. All animal handling and behavior sampling methods complied with the guidelines of the Institutional Animal Ethics Committee (Indian Institute of Science).

Behavior Sampling

The behavior of tagged animals was recorded using focal animal sampling in a repeated-measures design over their breeding lifespan. Using binoculars and a voice-recorder, during each sampling session, the switch from one behavioral state to another and every occurrence of selected behavioral events were continuously recorded. We recorded all the main display behavioral traits (e.g., headbob, pushup, gape, gular extension etc.), initially identified through previous work on *P. dorsalis* (Radder et al., 2005, 2006) and through preliminary observations at the study site (SD, unpublished data). We also recorded several behaviors which do not seem to be directly related to interacting with mates or competitors but likely related to maintaining body condition. These include foraging, moving (can be used to move toward resources, for thermoregulation, or to move away from predators) and alert behaviors (can be used for predator-detection); for a list of behaviors and their definitions, see Supplementary Table A. Male color was visually evaluated, classified as one of 8 mutually exclusive categories (states), and continuously monitored (**Figure 1**). Conspecifics within a 10 m radius were counted (once at the beginning, and subsequently, every 3–4 min during the session) and used to quantify two social contexts, namely the number of potential mates (females) and conspecific competitors (males) in the vicinity. Two ecological conditions, the presence/absence of predators and month (time during the breeding season), were recorded. The focal individual was followed for a minimum of 10 min and up to 30 min or till the individual disappeared from sight. Each focal session recording was later transcribed. For obtaining measures that are representative of the signaling behavior of an individual over the long-term, an individual was sampled regularly over its breeding lifespan. One to three focal sessions were conducted every month (not more than 1 session/day), over its breeding lifespan, until the animal was no longer seen at the study-site.

Quantifying Male Fitness

Since male fitness could not be directly quantified with parentage assignment using genetic analyses, we used two proxies of male fitness: (a) "females per day" and (b) "breeding tenure" (see below). Similar measures have been used as proxies of male reproductive success in reptilian studies (Ruby, 1984; Lappin and Husak, 2005). To estimate proxies of male fitness, tagged individuals were regularly monitored till they disappeared (presumed dead, Deodhar and Isvaran, 2017). Sheet rocks and frequently used perches were mapped using a GPS (Garmin eTrexH). Locations of all lizards, tagged and untagged, were regularly recorded every time a sheet rock was visited for behavioral observations, and during censuses (at least fortnightly during the breeding season, and monthly during the non-breeding season) carried out as part of a long-term monitoring study. These data provided information on (a) the duration (in days) for which a male was resident on the sheet rock (henceforth, tenure) and (b) monthly home ranges of known individuals, which were calculated by drawing 95% minimum convex polygons. Based on long-term observations, which show that adult male movement between sheet rocks is rare (among the 208 lizards tagged between 2010 and 2013, only 6 instances

FIGURE 1 | Photos of colors exhibited by males. Clockwise from top-left **(A)** pale **(B)** pale yellow **(C)** yellow **(D)** yellow ochre **(E)** orange **(F)** bright orange **(G)** crimson, and **(H)** fighting. To reduce the number of predictors, these 8 mutually exclusive states (see Supplementary Material Table A) of time spent in a given color were collapsed into 4 biologically meaningful levels, namely L1 (A+B), L2 (C+D), L3 (E+F+G), and "Fighting" colors.

of movement between sheet rocks were observed), adult males disappearing from a sheet rock were considered dead (Deodhar and Isvaran, 2017). Therefore, the measure of a male's tenure is likely to reflect his total adult lifespan. These data were used to calculate:

Females Per Day

This is an index of the number of mates that a male potentially had access to. Specifically, this proxy was calculated as the number of unique females present per day in a male's monthly home range, averaged over the months that a male was resident on the sheet rock. Using location data, we drew monthly 95% minimum convex polygons for each male, summed the number of unique females recorded in his monthly polygon during every observation session (behavioral session or census) in that month, and divided by the number of sessions/censuses during which that male's territory was surveyed. The estimates for the different months that a male was resident on the sheet rock were averaged to provide a lifetime "females per day" value for each male.

Breeding Tenure

The time for which a male was resident during the breeding season (May–Sep) alone was defined as the "breeding tenure" of a male. We assumed that the longer the breeding tenure the greater the access to potential mates. Since all males were followed till they disappeared from the study site (presumed dead) and since males typically experience only one breeding season, we obtained lifetime measures of breeding tenure.

Statistical Analyses

All analyses were carried out using R (Version 3.3.0) (R Core Team, 2016). For each focal sampling session, rates (counts per hour) of various behavioral events and proportions of time spent in various behavioral states were calculated (Supplementary Material Data sheet 4). To reduce the number of variables, the 8 mutually exclusive states of time spent in a given color were collapsed into 4 biologically meaningful levels (Pale:L1, Yellow:L2, Orange:L3 and "Fighting"; **Figure 1**, Supplementary Table A). To check for covariation in behaviors, we carried out a Principal Component Analysis (PCA) on selected, scaled behaviors (Supplementary Material Data Sheet 1). Prior to the PCA, we performed the Bartlett sphericity test and estimated the Keyser-Meyer-Olkin (KMO) measure to assess sampling adequacy (Budaev, 2010). The correlation matrix of behaviors is provided as Supplementary Material (Data sheets 5, 6) as recommended by Budaev (2010). We omitted rare behaviors (those seen in <20% of all focal sessions). To test for relationships of male behavior with social (number of mates and competitors in the vicinity) and ecological variables (season, predator-presence), we fitted linear mixed effects models, with composite behavioral variables (PC1a and PC2a, the first two principal components from the above PCA) as response variables (Supplementary Material Data Sheet 3). Since male behaviors loaded negatively on PC1a, we used (-PC1a) as the response variable for ease of interpretation (so that a large value of the response variable represented a higher rate/proportion of time spent in a state). The number of females (continuous) in the vicinity, number of males in the vicinity (continuous), season (factor with 6 levels, May–Oct) and predator presence (factor with two levels, present/absent) were included as fixed effects, and individual focal male ID as a random effect. Likelihood ratio tests were used to test the statistical significance of fixed effects. Following Nakagawa and Schielzeth (2013), we report both marginal and conditional R^2_{GLMM}, measures of the variance explained by the fixed effects and by the whole model (fixed and random effects), respectively. In order to compare the relative effects of each fixed effect, we report the change in the R^2_{GLMM} when each fixed effect is removed from the global model while retaining all remaining terms.

These mixed effects models represent a conservative test of the relationship between male behavior and predictors since we fitted only two models for the two composite behavioral variables. To supplement these analyses, we also fitted separate generalized linear mixed effects models (GLMMs) to examine the relationship of individual behaviors with social and ecological predictors. Depending on the nature of the individual response variables, suitable error structures were chosen. Firstly, for those behavioral events measured as rates and which were relatively rare (more than 40% of sampling sessions consisting of zeros) (viz. chase, crouch-shudder, change perch, forage: see Supplementary Table B), we used the occurrence of that event (present/absent) during the focal session as a binary response variable and used binomial error structure. Furthermore, for these rare behaviors, because of the large number of zeros, the effective degrees of freedom were relatively low. Therefore, we needed to reduce the number of parameters to be estimated in the statistical model, which was achieved by collapsing the levels for two of the predictor variables, viz. season and number of males in vicinity (this variable was chosen for collapsing over the number of females since the range in values was lower for the former rather than the latter). Thus, we included males in the vicinity as a categorical variable (present/absent), and season with a reduced number of levels (3 levels: May–Jun, Jul–Aug, Sep–Oct). Predator presence (present/absent) and number of females (continuous) were the other fixed effects in these models. Secondly, for behavioral events, measured as rates, which were common (<40% of sampling sessions consisting of zeros) (viz. headbob, move, pushup, reorient), we modeled the behavior as frequencies (number of counts per sampling session) and used negative binomial errors, and included the duration of the session (in seconds) as an offset to account for variation in sampling effort. For these behaviors, numbers of males and females in vicinity were included as continuous fixed effects and predator presence (present/absent) and season (6 levels, May:Oct) as categorical fixed effects. Thirdly, for modeling behaviors measured as proportion time spent in a state (viz. L1, L3), we used a quasibinomial error structure to account for overdispersion with the same fixed effects as those used for the common behavioral events. Individual ID was included as a random effect in all GLMMs.

Finally, we tested for the relationships of male traits with proxies of fitness. Since we were interested in individual-specific behavior unaffected by the immediate conditions experienced by an individual, we wished to obtain estimates of behavior after controlling for immediate social and ecological contexts. For this, we obtained adjusted behavioral rates and proportions of time spent in behavioral states by extracting random effects from the above GLMMs of individual behaviors. We first performed a PCA on adjusted behaviors to test for covariation. Since they showed strong covariation, we extracted the first two principal components (PC1b, PC2b). Next, we fitted linear models for each of the two proxies of male fitness, with composite behavioral variables (PC1b, PC2b) and additionally the log-transformed body size (measured at start of tenure) as predictors (Supplementary Material Data Sheet 2). We included male body size at the start of his tenure, since body size is known to affect male fitness in reptiles (Kingsolver and Raymond, 2008).

RESULTS

We tagged 138 males and obtained 101 focal observation sessions on 41 males (mean = 2.5 sessions/male, *SD* = 1.4, range = 1:6) over their breeding life span. All traits varied widely (Supplementary Table B). Within a focal session, males changed colors frequently, with much variation among males in the time spent in the different color states. Such a behavior of dynamic color change is rare among lizards.

Relationship of Male Behavior With Social and Ecological Context

In the PCA of behavioral traits, most of the display-related behaviors (headbob, pushup, crouch-shudder, proportion of time spent in orange color) co-varied closely and loaded strongly on the first PC axis (PC1a). That is, lizards with higher rates of headbob also performed crouch-shudder display and pushup more frequently and spent more time in orange color. Since this axis largely represents correlated display traits (factor loadings displayed in **Table 1**), we henceforth call it "composite display-index." Behaviors related to maintaining body condition (mainly foraging) strongly loaded on the second PC axis (PC2a; henceforth called "body-maintenance") (**Figure 2**, **Table 1**). These two components cumulatively explained 50% of the variation in the data. Bartlett sphericity test [$\chi^2_{(45)} = 350.68$, $p < 0.001$] and KMO measure (KMO = 0.60) confirmed that sampling was adequate. Modeling each of these two axes as a function of social and ecological contexts revealed that variation among focal sessions in composite display-index was most strongly related to the number of females in the vicinity during a session, and to a lesser extent, to predator presence (**Table 2**, refer to $\Delta R^2_{\mathrm{GLMM}}$ column). The composite display-index appeared to increase with the number of females in the vicinity (**Figure 3A**), indicating greater rates of displays and more time spent in orange color with more females in the vicinity. The display index appeared to decrease with predator presence (**Figure 3B**), suggesting overall reduced signaling in the presence of predators. The composite display-index was not consistently related to the number of males in the vicinity and varied marginally across months. Body-maintenance related behaviors appeared to decrease with predator presence (**Figure 3C**), varied marginally across months, but did not show clear relationships with either the number of females or males in the vicinity (**Table 2**).

Results of the Supplementary Models for individual behaviors closely supported those from analyses of composite behavioral variables (**Tables 3**, **4**). Most display traits (e.g., headbob, crouch-shudder, proportion of time spent in orange color) were positively related to the number of females in the focal male's vicinity and negatively to predator presence. Only two traits—L1 color (positively) and L3 color (negatively)—were related to the number of males in the vicinity.

Relationship of Basal Behavior With Male Fitness Proxies

PCA revealed that behaviors adjusted for immediate social and ecological contexts still co-varied (**Table 5**, Supplementary Figure A). Bartlett sphericity test [$\chi^2_{(45)} = 429.32$, $p < 0.001$) and KMO measure (KMO = 0.69) confirmed that sampling was adequate. Display-related and body-maintenance behaviors loaded strongly on the first and second PC axes (PC1b, PC2b), respectively, which together explained 47% of the variation in the data. Lifetime breeding tenure was substantially related to composite display-index (adjusted) ($R^2 = 0.26$; β[95% CI] = 38.05[13.69–62.42], $F_{(1,34)} = 10.07$, $P = 0.003$), but not detectably to body-maintenance (β[95% CI] = −16.69[−41.09 to 7.72], $F_{(1,34)} = 1.93$, $P = 0.173$) or size at start of tenure (β[95% CI] = −11.85[−160.33 to 136.64], $F_{(1,34)} = 0.02$, $P = 0.872$). Males that displayed more (i.e., higher values of composite display-index) had longer breeding tenures (**Figure 4A**). Females per day was consistently related to size at start of tenure ($R^2 = 0.34$; β[95% CI] = −4.26[−7.52 to 0.99], $F_{(1,16)} = 7.64$, $P = 0.014$), but not detectably to composite display-index (β[95% CI] = 0.19[−0.36 to 0.73], $F_{(1,16)} = 0.54$, $P = 0.474$) or body-maintenance (β[95% CI] = 0.06[−0.43 to 0.56], $F_{(1,16)} = 0.08$, $P = 0.786$). Males that began their tenure at a smaller size appeared to have home ranges in areas with higher female densities (**Figure 4B**).

TABLE 1 | Factor loadings of common behaviors in a Principal Component Analysis of common behaviors displayed across focal animal sampling sessions (n = 101) showing strong covariation in behaviors during a given session.

Behavior (code)	PC1a	PC2a
Chase (ch)	0.9474	−0.4886
Crouch-shudder (cns)	1.1711	−0.4670
Change perch (cp)	1.1377	0.1608
Forage (f)	0.3148	1.4994
Headbob (h)	1.2159	−0.0619
Move (m)	1.3368	0.7698
Pushup (p)	1.2045	−0.1937
Reorient (s)	1.0493	0.7479
Pale color (L1)	−0.6386	0.2214
Orange color (L3)	1.0051	−1.0040

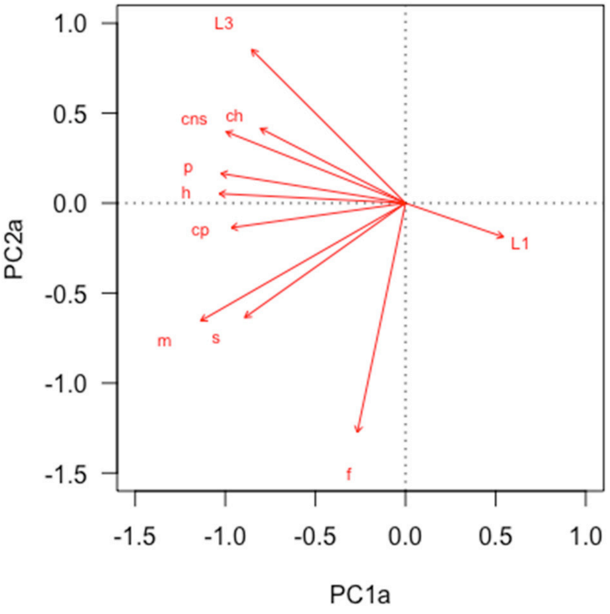

FIGURE 2 | Principal Component Analysis of common behaviors across focal animal sampling sessions (n = 101) revealed strong covariation. Text-labels at the tip of each vector indicate behaviors (ch:Chase, cns:Crouch-shudder, cp:Change perch, f:Forage, h:Headbob, m:Move, p:Pushup, s:Reorient, L1:Pale color, L3:Orange color). See **Table 1** for factor loadings.

TABLE 2 | Results from modeling composite behavioral variables, composite display-index, and body-maintenance, as a function of social (number of females and males in vicinity) and ecological (month and presence of predator) contexts using LMMs ($n = 101$ focal sessions).

Term	PC1a: Composite display index						PC2a: Body-maintenance					
	Estimate	**SE**	χ^2	*df*	*P*	ΔR^2_{GLMM}	**Estimate**	**SE**	χ^2	*df*	*P*	ΔR^2_{GLMM}
Intercept (Month:May; Predator: Absent)	−0.35	0.17					0.07	0.18				
Number of females	0.20	0.05	14.24	1	0.0001	−0.12	0.01	0.06	0.01	1	0.910	<−0.01
Number of males	0.04	0.07	0.23	1	0.634	<−0.01	0.13	0.07	3.11	1	0.078	−0.03
Predator-presence: Present	−0.34	0.15	4.85	1	0.028	−0.04	−0.35	0.16	5.48	1	0.019	−0.05
Month (overall effect)			11.05	5	0.050	−0.08			10.39	5	0.065	−0.08
Month: June	0.03	0.17					−0.08	0.17				
Month: July	−0.35	0.22					−0.61	0.23				
Month: August	0.36	0.23					−0.00	0.25				
Month: September	0.42	0.26					0.16	0.26				
Month: October	0.03	0.34					−0.16	0.33				

Parameter estimates, standard errors, χ^2 and P-values from likelihood ratio tests of fixed effects are shown. The R^2_{GLMM} [marginal, conditional] values for the full model for composite display-index are [0.17, 0.22], while those for body-maintenance are [0.14, 0.32], respectively. The ΔR^2_{GLMM} (marginal) represents the change in the R^2_{GLMM} values upon dropping a predictor from the full model (R^2_{GLMM} Reduced model-R^2_{GLMM} Full model) and represents the relative importance of that predictor in the model. A more negative value indicates a greater reduction in model fit associated with dropping that predictor from the model (i.e., greater relative importance). See Methods section for model details.

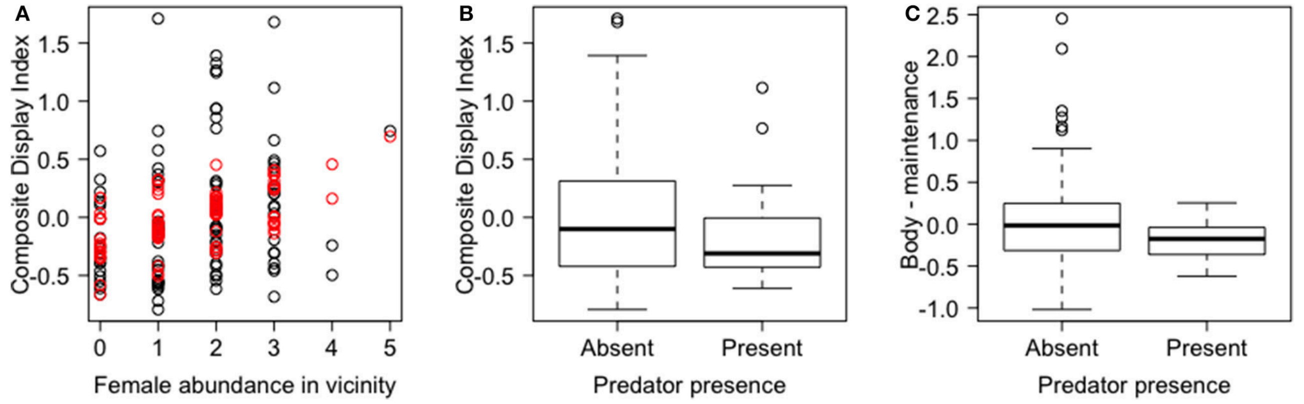

FIGURE 3 | Relationships of composite behavioral variables (Principal Component axes) with social and ecological contexts. The main relationships detected in the linear mixed effects models of the two composite behavioral variables are shown. **(A)** Composite display-index, i.e., PC1a, (observed—black and predicted-red) is positively related to female abundance in the vicinity; **(B)** composite display-index is negatively related to predator presence; and **(C)** body-maintenance, i.e., PC2a, is negatively related to predator presence. Box plots show median and inter-quartile range.

DISCUSSION

We found that males regularly signal to conspecifics using multiple closely correlated traits, under natural ecological and social contexts. These traits appear to be primarily directed toward females, and to contribute toward male fitness. Our findings from a wild population provide rare evidence that these signaling traits are biologically relevant and appear to be influenced by multiple selection pressures.

Variation in Male Traits

Males typically used multiple behavioral traits while signaling, of which some (e.g., headbobs, pushups) were more common than others (e.g., gular extension, mounting). These stereotypical behaviors have been documented in other lizard species (Cowles, 1956; LeBas and Marshall, 2000; Radder et al., 2006). Apart from body postures and movements, *P. dorsalis* males were also observed dynamically changing their coloration from striking and conspicuous color patterns to paler and duller ones. Individual males modulated the color of their dorsal strip within a few seconds, with the lateral and ventral sides maintaining a dark color (see **Figure 1**). Additionally, during certain close-range male-male interactions, males showed a radically different color pattern ("Fighting" coloration), with red lateral and ventral sides and a yellow dorsal strip (**Figure 1**). Although such "dynamic color change" behavior has previously

TABLE 3 | Relationship of individual male behaviors (rates) with immediate social and ecological context **.

Term	Chase*					Crouch-shudder*					Change perch*					Forage*				
	Est.	**SE**	**χ^2**	***df***	***P***	**Est.**	**SE**	**χ^2**	***df***	***P***	**Est.**	**SE**	**χ^2**	***df***	***P***	**Est.**	**SE**	**χ^2**	***df***	***P***
Intercept (Month: May–Jun; Predator: Absent)	−2.91	0.70				−1.57	0.56				0.31	0.69				0.57	0.50			
Number of females	0.63	0.25	7.19	1	0.007	0.46	0.22	4.69	1	0.030	0.21	0.26	0.60	1	0.437	0.06	0.21	0.09	1	0.770
Number of males	0.91	0.53	3.05	1	0.081	−0.25	0.49	0.26	1	0.610	−0.51	0.57	0.77	1	0.379	−0.54	0.47	1.35	1	0.246
Predator–presence:Present	−0.03	0.71	0.00	1	0.968	−1.12	0.82	2.24	1	0.134	0.51	0.78	0.41	1	0.521	−2.07	0.82	8.63	1	0.003
Month (overall effect)			0.20	2	0.905			0.14	2	0.933			1.28	2	0.528			12.60	2	0.002
Month: Jul–Aug	0.17	0.67				0.17	0.60				−0.46	0.73				−2.16	0.71			
Month: Sep–Oct	0.39	0.94				−0.15	0.88				0.66	1.03				−0.20	0.76			

Term	Headbob#					Move#					Pushup#					Reorient#				
	Est.	**SE**	**χ^2**	***df***	***P***	**Est.**	**SE**	**χ^2**	***df***	***P***	**Est.**	**SE**	**χ^2**	***df***	***P***	**Est.**	**SE**	**χ^2**	***df***	***P***
Intercept (Month: May; Predator: Absent)	−4.90	0.37				−6.14	0.36				−5.76	0.48				−5.89	0.26			
Number of females	0.30	0.12	5.95	1	0.015	0.40	0.12	10.7	1	0.001	0.29	0.16	3.42	1	0.065	0.09	0.08	1.41	1	0.235
Number of males	0.02	0.15	0.01	1	0.920	0.16	0.14	1.43	1	0.232	0.05	0.19	0.07	1	0.788	0.19	0.10	3.32	1	0.069
Predator presence: Present	−0.86	0.32	6.55	1	0.011	−0.71	0.32	4.35	1	0.037	0.04	0.42	0.01	1	0.929	−0.60	0.24	5.88	1	0.015
Month (overall effect)			11.86	5	0.037			12.47	5	0.029			4.80	5	0.440			18.86	5	0.002
Month: Jun	0.28	0.34				−0.02	0.33				−0.27	0.44				−0.20	0.24			
Month: Jul	−1.20	0.47				−0.97	0.47				−0.77	0.61				−1.50	0.41			
Month: Aug	0.34	0.50				0.67	0.48				0.25	0.61				0.05	0.34			
Month: Sep	0.49	0.52				0.92	0.50				0.73	0.67				0.13	0.37			
Month: Oct	0.35	0.64				−0.01	0.66				0.44	0.88				−1.12	0.51			

*Results from modeling individual male behavior rates as a function of social (number of females and males in vicinity) and ecological (month and presence of predator) context using GLMMs. *, "Month" with 3 levels (May–Jun, Jul–Aug, Sep–Oct) and "Male" as categorical (present/absent). #, "Month" with 6 levels (May to Oct) and "Male" as continuous variable.*

***, Although there are many null hypothesis significance tests reported in **Tables 2–4**, we did not perform table-wise multiple comparisons corrections because the utility of this is still debated (e.g., Feise, 2002; Moran, 2003). We follow the principle that p-values are guidelines and look for larger concordance in results than at the meaning of individual p values. That is, we examine whether the patterns in individual behaviors broadly support the conservative test done on composite behavioral variables (PC1a: composite display-index and PC2a: body-maintenance). Please note that the main tests in the paper are carried out as conservatively as possible (on composite behaviors rather than individual behaviors) so that we arrive at inferences that are robust.*

TABLE 4 | Relationship of male coloration with immediate social and ecological context.

Term	Pale (L1)					Yellow (L2)					Orange (L3)				
	Est.	SE	χ^2	*df*	*P*	Est.	SE	χ^2	*df*	*P*	Est.	SE	χ^2	*df*	*P*
Intercept (Month: May; Predator: Absent)	−1.24	0.67				0.68	0.51				−2.14	0.70			
Number of females	−0.24	0.24	1.08	1	0.298	−0.20	0.16	1.53	1	0.216	0.37	0.19	3.84	1	0.050
Number of males	0.67	0.29	5.33	1	0.021	−0.17	0.22	0.57	1	0.449	−0.68	0.30	5.82	1	0.016
Predator presence: Present	−1.08	0.89	1.80	1	0.180	0.43	0.46	0.87	1	0.352	−0.58	0.54	1.19	1	0.276
Month (overall effect)			4.75	5	0.448			12.25	5	0.031			12.06	5	0.034
Month: Jun	−0.62	0.63				0.22	0.47				0.74	0.65			
Month: Jul	0.36	0.78				−1.17	0.68				1.33	0.77			
Month: Aug	−0.90	1.03				−1.36	0.72				2.16	0.85			
Month: Sep	−0.88	1.28				−0.12	0.76				1.12	0.90			
Month: Oct	−3.04	3.15				−0.49	0.81				−15.62	1580.43			

Results from modeling male coloration (proportion of time spent in a given color) as a function of social (number of females and males in vicinity) and ecological (month and presence of predator) context using GLMMs.

TABLE 5 | Factor loadings from the Principal Component Analysis checking for covariation in adjusted behavior across males.

Behavior (code)	PC1b	PC2b
Chase (ch)	1.0678	−0.0047
Crouch-shudder (cns)	0.5330	0.7467
Change perch (cp)	0.5203	−0.6223
Forage (f)	−0.3465	−0.8051
Headbob (h)	0.9568	−0.0284
Move (m)	0.5483	−0.5650
Pushup (p)	1.1150	−0.3430
Reorient (s)	0.9049	−0.5583
Pale color (L1)	−0.4348	−0.7906
Orange color (L3)	0.9741	0.5470

Behaviors related to male display appear to load strongly on the first component (PC1b), while those related to body-maintenance on the second component (PC2b).

been reported in a few other species (Stuart-Fox and Moussalli, 2008; Kindermann et al., 2013; Teyssier et al., 2015) and recently in *P. dorsalis* (Batabyal and Thaker, 2017) there is relatively little information on this behavior from wild populations. We found that, like with other signals (e.g., headbob, pushup), males varied widely in the time they spent displaying in different coloration patterns.

What Maintains These Signaling Traits?

Male signaling behavior was dynamic and apparently sensitive to the costs and benefits associated with immediate social and ecological conditions. Our results suggest that the main benefits from signaling are related to attracting mates rather than to modulating male-male competition, and that predation risk is an important cost.

The frequency of most signaling traits increased with an increasing number of females in the vicinity, suggesting that they function in mate-choice. Similar patterns of males directing signals toward females (broadcasting and courtship), rather than toward males, have been reported from a few other lizards [collared lizard *Crotyphytus collaris* (Baird, 2013), brown anole *Anolis sagrei* (Driessens et al., 2014)]. Most signaling behaviors were not consistently associated with male abundance in the vicinity suggesting that their primary role may not lie in intrasexual competition. However, there was a weak relationship between the time spent in pale (+ve correlation) and orange (−ve correlation) colors and number of males, suggesting that the pale color pattern could be involved in male-male competition. Given that males can display only one color pattern at a time, if pale coloration is indeed directed toward males, and orange coloration toward females, this would indicate an interesting trade-off between intrasexual and intersexual signaling that could be pursued in future studies. Future work that experimentally simulates close encounters with competitors on male territories would help clarify the role of signaling traits in male-male competition.

Why Multiple Traits?

A striking result of this study is that males typically displayed using multiple signals, these multiple male signals covaried strongly, and increased simultaneously with increasing female abundance. These findings have implications for hypotheses of the maintenance of multiple signals. The clear covariation in signals in our study has rarely been reported (Candolin, 2003; Chaine and Lyon, 2015). The few previous studies that have explicitly examined for correlations in traits (Bro-Jørgensen and Dabelsteen, 2008; Chaine and Lyon, 2015; Ferrer et al., 2015) have mostly found weak or limited correlation in traits (but see Girard et al., 2015; Hegyi et al., 2015). The covariation of the main signaling traits in *P. dorsalis* and their relationship with female abundance suggests that the signals are redundant, and are perhaps maintained in

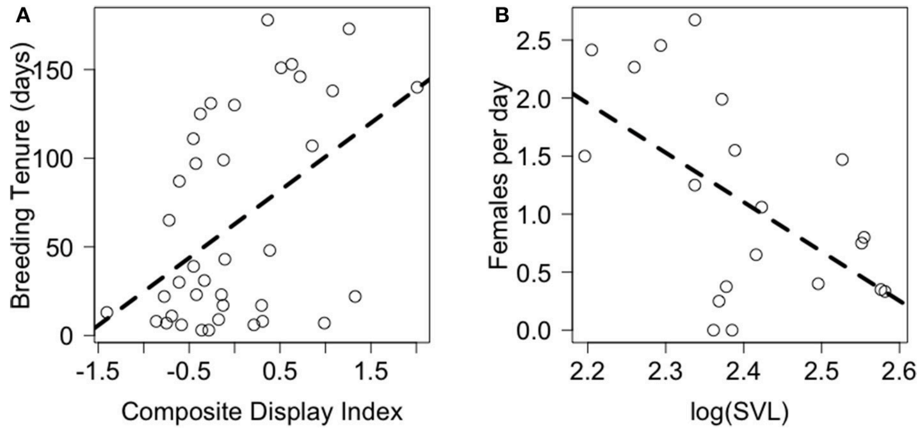

FIGURE 4 | Relationship of proxies of male fitness, namely **(A)** breeding tenure and **(B)** females per day with **(A)** composite display-index and **(B)** body size at start of tenure, respectively. Dashed lines represent model predictions.

the population because multiple redundant signals facilitate quicker and more accurate assessment of male phenotypic quality by females. Further work on the costs of these signals, the information they convey, and the response of receivers is needed to resolve how many of these signals are maintained because they are costly honest indicators (Moller and Pomiankowski, 1993; Johnstone, 1996) vs. non-informative low-cost signals that improve detectability and/or discriminability (Rowe, 1999).

Alternatively, each of these signaling traits could represent a different component of the male's quality and therefore be maintained as "multiple messages." However, the strong correlations in these traits suggest that the different male-quality components are then strongly correlated. If this is the case, selection should result in the reduction of multiple signals to a single or a few traits that represent the correlated quality traits. Of the range of traits measured, a color trait (viz. time spent in pale color) showed a weak relationship with number of conspecific males in vicinity, suggesting that some of the traits could be maintained as multiple messages directed to multiple receivers. A study of *Taurotragus oryx* similarly suggested that both multiple message and redundant signal mechanisms might be involved in the maintenance of multiple signals (Bro-Jørgensen and Dabelsteen, 2008).

To summarize, the strong correlation between most of the traits measured suggests that these traits are maintained because of the greater effectiveness of multiple redundant traits in communicating content compared with a single trait. Further work on the information conveyed by these signals and the response of receivers to these signals is needed to confirm that these signals are redundant and to obtain a detailed understanding of the mechanisms maintaining these signals. Regardless of whether they are redundant signals, or whether they might represent multiple, correlated aspects of male quality, we find that these multiple traits appear to be primarily maintained in this population through sexual selection via female choice. Our findings highlight the insights that can be gained from comprehensively measuring the communication repertoire under multiple contexts.

Apart from social factors, we found that predation likely contributes toward maintaining variation in signals. None of the displays increased in the presence of predators suggesting that in *P. dorsalis*, these traits are not directed toward predators as a means to deter predation attempts. Rather, several displays appeared to reduce in the presence of predators, which suggests that these signals carry the cost of increased predation risk. Examining the evidence from other lizards, studies of *Anolis* lizards report contrasting results for the role of predation on male signals (Driessens et al., 2014). More generally, there is evidence from a wide range of taxa that predation risk is a common cost of conspicuous sexually-selected display traits (Tuttle and Ryan, 1981; Endler, 1992; Mougeot and Bretagnolle, 2000; Jones et al., 2002; Godin and McDonough, 2003; Stuart-Fox et al., 2003; Husak et al., 2006; Halfwerk et al., 2014).

Effect of Male Traits on Fitness

Our findings suggest that signaling can have important fitness consequences. Males that signaled more had longer breeding tenures. We assume that longer tenures are associated with increased mating opportunities. Previous studies, mostly covering a part of the lifespan, have also found that signaling has fitness consequences and is likely to experience strong selection (Bradbury and Vehrencamp, 1998; Girard et al., 2015); however, information on the relationship between signaling and lifetime measures of reproductive success is scarce.

Further, we found that males that were smaller in size at the start of their tenure were able to establish a territory in areas with higher female densities. This was unexpected, since we predicted that larger males would have their territories in female-dense areas. Since body size is correlated with age in many reptiles, one possible explanation is that individuals who are able to begin defending territories when younger are of higher quality, and correspondingly able to defend territories in female-dense areas, compared with males who establish territories when older (larger).

CONCLUSIONS

By tracking known individuals over their breeding lifespans in a wild population and comprehensively studying the signaling repertoire of breeding males, we found that multiple selection pressures (namely, intersexual selection and predation risk) appeared to affect male signaling traits. Most signaling traits appeared to be strongly correlated and directed toward females, providing support for the redundant signal hypothesis. A few traits seemed to be directed at conspecific males, providing limited support for the multiple message hypothesis. Finally, we found that the strongly correlated set of male behavioral signals, together with a morphological trait, may influence lifetime reproductive success, highlighting the biological relevance of these signaling traits.

DATA AVAILABILITY

Data and codes of statistical analyses are provided in the Supplementary Material. All descriptions of data sheets are included in the code.

AUTHOR CONTRIBUTIONS

SD and KI were equally involved in designing the study, performing statistical analyses, and writing the manuscript. SD performed all fieldwork.

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SUPPLEMENTARY MATERIAL

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REFERENCES

Andersson, S., and Pryke, S. R., Ornborg, J., Lawes, M. J., and Andersson, M. (2002). Multiple receivers, multiple ornaments, and a trade-off between agonistic and epigamic signaling in a widowbird. *Am. Nat.* 160, 683–691. doi: 10.1086/342817

Baird, T. A. (2013). Male collared lizards, *Crotaphytus collaris* (*Sauria: Crotaphytidae*), signal females by broadcasting visual displays. *Biol. J. Linn. Soc.* 108, 636–646. doi: 10.1111/bij.12003

Batabyal, A., and Thaker, M. (2017). Signalling with physiological colours: high contrast for courtship but speed for competition. *Anim. Behav.* 129, 229–236. doi: 10.1016/j.anbehav.2017.05.018

Bókony, V., Lendvai, Á. Z., and Liker, A. (2006). Multiple cues in status signalling: the role of wingbars in aggressive interactions of male house sparrows. *Ethology* 112, 947–954. doi: 10.1111/j.1439-0310.2006.01246.x

Bradbury, J. W., and Vehrencamp, S. L. (1998). Principles of *Animal Communication*. Sunderland, MA: Sinauer Associates Inc.

Brodie, E. D. Jr. (1977). Salamander antipredator postures. *Copeia* 3, 523–535. doi: 10.2307/1443271

Bro-Jørgensen, J. (2010). Dynamics of multiple signalling systems: animal communication in a world in flux. *Trends Ecol. Evol.* 25, 292–300. doi: 10.1016/j.tree.2009.11.003

Bro-Jørgensen, J., and Dabelsteen, T. (2008). Knee-clicks and visual traits indicate fighting ability in eland antelopes: multiple messages and back-up signals. *BMC Biol.* 6:47. doi: 10.1186/1741-7007-6-47

Budaev, S. V. (2010). Using principal components and factor analysis in animal behaviour research: caveats and guidelines. *Ethology* 116, 472–480. doi: 10.1111/j.1439-0310.2010.01758.x

Candolin, U. (2003). The use of multiple cues in mate choice. *Biol. Rev.* 78, 575–595. doi: 10.1017/S1464793103006158

Caro, T. M. (1986). The functions of stotting in Thomson's gazelles: some tests of the predictions. *Anim. Behav.* 34, 663–684.

Chaine, A. S., and Lyon, B. E. (2015). Signal architecture: temporal variability and individual consistency of multiple sexually selected signals (T Williams, Ed). *Funct. Ecol.* 29, 1178–1188. doi: 10.1111/1365-2435.12410

Cowles, R. B. (1956). Notes on natural history of a South African agamid lizard. *Herpetologica* 12, 297–302.

Deodhar, S., and Isvaran, K. (2017). Breeding phenology of *Psammophilus dorsalis*: patterns in time, space and morphology. *Curr. Sci.* 113:2120. doi: 10.18520/cs/v113/i11/2120-2126

Driessens, T., Vanhooydonck, B., and Van Damme, R. (2014). Deterring predators, daunting opponents or drawing partners? Signaling rates across diverse contexts in the lizard Anolis sagrei. *Behav. Ecol. Sociobiol.* 68, 173–184. doi: 10.1007/s00265-013-1669-4

Endler, J. A. (1992). Signals, signal conditions, and the direction of evolution. *Am. Nat.* 139, S125–S153. doi: 10.1086/285308

Feise, R. J. (2002). Do multiple outcome measures require p-value adjustment? *BMC Med. Res. Methodol.* 2:8. doi: 10.1186/1471-2288-2-8

Ferrer, E. S., García-Navas, V., Bueno-Enciso, J., José Sanz, J., and Ortego, J. (2015). Multiple sexual ornaments signal heterozygosity in male blue tits. *Biol. J. Linn. Soc.* 115, 362–375. doi: 10.1111/bij.12513

Fisher, M., and Muth, A. (1989). A technique for permanently marking lizards. *Herpetol. Rev.* 20, 45–46.

Girard, M. B., Elias, D. O., and Kasumovic, M. M. (2015). Female preference for multi-modal courtship: multiple signals are important for male mating success in peacock spiders. *Proc. R. Soc. B Biol. Sci.* 282:20152222. doi: 10.1098/rspb.2015.2222

Godin, J.-G. J., and McDonough, H. E. (2003). Predator preference for brightly colored males in the guppy: a viability cost for a sexually selected trait. *Behav. Ecol.* 14, 194–200. doi: 10.1093/beheco/14.2.194

Halfwerk, W., Dixon, M. M., Ottens, K. J., Kristina J., Taylor, R. C., Ryan, M. J., et al. (2014). Risks of multimodal signaling: bat predators attend to dynamic motion in frog sexual displays. *J. Exp. Biol.* 217, 3038–3044. doi: 10.1242/jeb.107482

Hamilton, D. G., Whiting, M. J., and Pryke, S. R. (2013). Fiery frills: carotenoid-based coloration predicts contest success in frillneck lizards. *Behav. Ecol.* 24, 1138–1149. doi: 10.1093/beheco/art041

Hamilton, P. S., and Sullivan, B. K. (2005). Female mate attraction in ornate tree lizards, *Urosaurus ornatus*: a multivariate analysis. *Anim. Behav.* 69, 219–224. doi: 10.1016/j.anbehav.2004.03.011

Hebets, E. A., and Papaj, D. R. (2005). Complex signal function: developing a framework of testable hypotheses. *Behav. Ecol. Sociobiol.* 57, 197–214. doi: 10.1007/s00265-004-0865-7

Hegyi, G., Laczi, M., Nagy, G., Szász, E., Kötél, D., Török, J., et al. (2015). Stable correlation structure among multiple plumage colour traits: can they work as a single signal? *Biol. J. Linn. Soc.* 114, 92–108. doi: 10.1111/bij.12412

Husak, J. F., Macedonia, J. M., Fox, S. F., and Sauceda, R. C. (2006). Predation cost of conspicuous male coloration in collared Lizards (*Crotaphytus collaris*): an experimental test using clay-covered model lizards. *Ethology* 112, 572–580. doi: 10.1111/j.1439-0310.2005.01189.x

Johnstone, R. A. (1996). Multiple displays in animal communication: "backup signals" and "multiple messages." *Philos. Trans. R. Soc. B Biol. Sci.* 351, 329–338.

Jones, G., Barabas, A., Elliot, W., and Stuart, P. (2002). Female greater wax moths reduce sexual display behavior in relation to the potential risk of predation by echolocating bats. *Behav. Ecol.* 13, 375–380. doi: 10.1093/beheco/13.3.375

Kindermann, C., Narayan, E. J., Wild, F., Wild, C. H., and Hero, J. M. (2013). The effect of stress and stress hormones on dynamic colour-change in a sexually dichromatic Australian frog. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 165, 223–227. doi: 10.1016/j.cbpa.2013.03.011

Kingsolver, J. G., and Raymond, H. M. (2008). Size, temperature, and fitness: three rules. *Evol. Ecol. Res.* 10, 251–268. Available online at: http://www.evolutionary-ecology.com/abstracts/v10/2242.html

Lappin, A. K., and Husak, J. F. (2005). Weapon performance, not size, determines mating success and potential reproductive output in the collared lizard (*Crotaphytus collaris*). *Am. Nat.* 166, 426–436. doi: 10.1086/432564

LeBas, N. R., and Marshall, N. J. (2000). The role of colour in signalling and male choice in the agamid lizard *Ctenophorus ornatus*. *Proc. R. Soc. B Biol. Sci.* 267, 445–452. doi: 10.1098/rspb.2000.1020

Loyau, A., Jalme, M. S., and Sorci, G. (2005). Intra- and Intersexual Selection for Multiple Traits in the Peacock (*Pavo cristatus*). *Ethology* 111, 810–820. doi: 10.1111/j.1439-0310.2005.01091.x

Marchetti, K. (1998). The evolution of multiple male traits in the yellow-browed leaf warbler. *Anim. Behav.* 55, 361–376. doi: 10.1006/anbe.1997.0586

Martín, J., and López, P. (2009). Multiple color signals may reveal multiple messages in male Schreiber's green lizards, *Lacerta schreiberi*. *Behav. Ecol. Sociobiol.* 63, 1743–1755. doi: 10.1007/s00265-009-0794-6

Moller, A. P., and Pomiankowski, A. (1993). Why have birds got multiple sexual ornaments? *Behav. Ecol. Sociobiol.* 32, 167–176. doi: 10.1007/BF00173774

Moran, M. D. (2003). Arguments for rejecting the sequential bonferroni in ecological studies. *Oikos* 100, 403–405. doi: 10.1034/j.1600-0706.2003.12010.x

Mougeot, F., and Bretagnolle, V. (2000). Predation risk and moonlight avoidance in nocturnal seabirds. *J. Avian Biol.* 31, 376–386. doi: 10.1034/j.1600-048X.2000.310314.x

Nakagawa, S., and Schielzeth, H. (2013). A general and simple method for obtaining R-squared values from generalized linear mixed-effects models (RB O'Hara, Ed). *Methods Ecol. Evol.* 4, 133–142. doi: 10.1111/j.2041-210x.2012.00261.x

Plasman, M., Reynoso, V. H., Nicolás, L., and Torres, R. (2015). Multiple colour traits signal performance and immune response in the Dickerson's collared lizard *Crotaphytus dickersonae*. *Behav. Ecol. Sociobiol.* 69, 765–775. doi: 10.1007/s00265-015-1892-2

Qvarnstrom, A. (1997). Experimentally increased badge size increases male competition and reduces male parental care in the collared flycatcher. *Proc. R. Soc. B Biol. Sci.* 264, 1225–1231. doi: 10.1098/rspb.1997.0169

Radder, R. S., Saidapur, S. K., and Shanbhag, B. A. (2005). Population density, microhabitat use and activity pattern of the Indian rock lizard, *Psammophilus dorsalis* (Agamidae). *Curr. Sci.* 89, 560–566. Available online at: http://www.currentscience.ac.in/Downloads/article_id_089_03_0560_0566_0.pdf

Radder, R. S., Saidapur, S. K., Shine, R., and Shanbhag, B. A. (2006). The language of lizards: interpreting the function of visual displays of the Indian rock lizard, *Psammophilus dorsalis* (Agamidae). *J. Ethol.* 24, 275–283. doi: 10.1007/s10164-006-0192-8

R Core Team (2016). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Rek, P., and Magrath, R. D. (2016). Multimodal duetting in magpie-larks: how do vocal and visual components contribute to a cooperative signal's function? *Anim. Behav.* 117, 35–42. doi: 10.1016/j.anbehav.2016.04.024

Roberts, J. A., Taylor, P. W., and Uetz, G. W. (2006). Consequences of complex signaling: predator detection of multimodal cues. *Behav. Ecol.* 18, 236–240. doi: 10.1093/beheco/arl079

Rowe, C. (1999). Receiver psychology and the evolution of multicomponent signals. *Anim. Behav.* 58, 921–931. doi: 10.1006/anbe.1999.1242

Ruby, D. E. (1984). Male breeding success and differential access to females in *Anolis carolinensis*. *Herpetologica* 40, 272–280.

Rundus, A. S., Sullivan-Beckers, L., Wilgers, D. J., and Hebets, E. A. (2011). Females are choosier in the dark: environment-dependent reliance on courtship components and its impact on fitness. *Evolution* 65, 268–282. doi: 10.1111/j.1558-5646.2010.01125.x

Smith, C. L., and Evans, C. S. (2008). Multimodal signaling in fowl, *Gallus gallus*. *J. Exp. Biol.* 211, 2052–2057. doi: 10.1242/jeb.017194

Stuart-Fox, D., and Moussalli, A. (2008). Selection for social signalling drives the evolution of chameleon colour change (FBM de Waal, Ed). *PLoS Biol.* 6:e25. doi: 10.1371/journal.pbio.0060025

Stuart-Fox, D., Moussalli, A., Marshall, N. J., and Owens, I. P. F. (2003). Conspicuous males suffer higher predation risk: visual modelling and experimental evidence from lizards. *Anim. Behav.* 66, 541–550. doi: 10.1006/anbe.2003.2235

Teyssier, J., Saenko, S. V., van der Marel, D., and Milinkovitch, M. C. (2015). Photonic crystals cause active colour change in chameleons. *Nat. Commun.* 6:6368. doi: 10.1038/ncomms7368

Tuttle, M. D., and Ryan, M. J. (1981). Bat predation and the evolution of frog vocalizations in the neotropics. *Science* 214, 677–678. doi: 10.1126/science.214.4521.677

Zuk, M., Ligon, J. D., and Thornhill, R. (1992). Effects of experimental manipulation of male secondary sex characters on female mate preference in red jungle fowl. *Anim. Behav.* 44, 999–1006. doi: 10.1016/S0003-3472(05)80312-4

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REVIEW
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Multimodal Aposematic Signals and Their Emerging Role in Mate Attraction

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Chemically defended animals often display conspicuous color patterns that predators learn to associate with their unprofitability and subsequently avoid. Such animals (i.e., aposematic), deter predators by stimulating their visual and chemical sensory channels. Hence, aposematism is considered to be "multimodal." The evolution of warning signals (and to a lesser degree their accompanying chemical defenses) is fundamentally linked to natural selection by predators. Lately, however, increasing evidence also points to a role of sexual selection shaping warning signal evolution. One of the species in which this has been shown is the wood tiger moth, *Arctia plantaginis*, which we here put forward as a promising model to investigate multimodality in aposematic and sexual signaling. *A. plantaginis* is an aposematic diurnal moth which exhibits sexually dimorphic coloration as well as sex-limited polymorphism in part of its range. The anti-predator function of its coloration and, more recently, its chemical defenses (even when experimentally decoupled from the visual signals), has been well-demonstrated. Interestingly, recent studies have revealed differences between the two male morphs in mating success, suggesting a role of coloration in mate choice or attraction, and providing a possible explanation for its sexual dimorphism in coloration. Here, we: (1) review the lines of evidence showing the role of predation pressure and sexual selection in the evolution of multimodal aposematic signals in general, and in the wood tiger moth in particular; (2) establish gaps in current research linking sexual selection and predation as selective pressures on aposematic signals by reviewing a sample of the literature published in the last 30 years; (3) highlight the need of identifying suitable systems to address simultaneously the effect of natural and sexual selection on multimodal aposematic signals; and (4) propose directions for future research to test how aposematic signals can evolve under natural and sexual selection.

Keywords: warning coloration, multimodal signals, predator-prey interactions, sexual selection, chemical signals, signal variation

INTRODUCTION

Animals can communicate their quality to potential mates or predators with different types of signals (Maynard Smith and Harper, 2003). Because signals may be targeted to different receivers, the multiple functions can sometimes lead to a conflict between natural and sexual selection, which imposes limitations on signal evolution. For instance, in Darwin and Fisher's sexual selection theories (Darwin, 1869; Fisher, 1930), some traits can be favored by sexual selection, such as the vivid body colors on a male guppy, *Poecilia reticulata* (Endler, 1988b; **Figure 1A**), but the evolution of these conspicuous ornaments may be constrained by the individual's survival, as they are also easier to detect by predators or parasites (Endler, 1988b; Kotiaho et al., 1998; Zuk and Kolluru, 1998; Lindström et al., 2005). Likewise, females of the Túngara frog, *Engystomops pustulosus*, prefer male mating calls of increased complexity which, in turn, are easier to detect and locate by bats (Ryan et al., 1982; **Figure 1C**); and females of the wolf spider *Hygrolycosa rubrofasciata* (**Figure 1D**) prefer males that drum their abdomen against the dry leaves at higher rates (Parri et al., 1997), which can lead to increased predation risk (Kotiaho et al., 1998). However, if different elements of the signal are targeted to a different receiver or evoke different responses, then they can evolve despite being the subject of both selective factors (Endler, 1992; **Figure 1**). That is the case in the dorsal and ventral markings in the wings of butterflies of the genus *Bicyclus* (Oliver et al., 2009), such as *B. Anynana* (**Figure 1B**). While the eyespots on the ventral side of their wings deter predators (Lyytinen et al., 2004), the UV reflection of the dorsal markings signal potential mates (Robertson and Monteiro, 2005).

One way to increase the efficacy of a particular signal is to stimulate multiple sensory modalities of the receiver simultaneously (Partan and Marler, 1999). This type of multimodal signals are used in the aposematic displays that defended organisms use to advertise their unprofitability (e.g., toxicity, unpalatability, or physical defenses such as spines) to potential predators (Poulton, 1890; Cott, 1940; Edmunds, 1974; Ruxton et al., 2004; Rojas et al., 2015b). Multimodal signals are also common in sexual communication where males can advertise their quality via multiple cues in multiple sensory channels (Bradbury and Vehrencamp, 2011). More recently, it has become evident that certain visual components, such as bright color patterns, in multimodal displays may have a dual function both as aposematic and sexual signals (Cummings and Crothers, 2013). In contrast, much less information exists on whether or not secondary defenses could also have a dual function in both chemical communication to potential predators and potential mates (Conner et al., 1981).

Here, we: (1) review the multiple lines of evidence showing both how predation pressure has shaped the evolution of multimodal aposematic signals, as well as the less studied role of sexual selection in warning color evolution; (2) establish the gaps in current studies linking sexual selection and predation as selective pressures on the warning displays of aposematic species, by reviewing a sample of the literature published over the last 30 years; (3) point out the need to identify representative model systems from different taxonomic groups where both

FIGURE 1 | Examples of organisms whose signals are under the influence of both sexual and natural selection. **(A)** Trinidadian guppy, *Poecilia reticulata*. Females prefer signals that are also an easier target for predators; **(B)** Squinting Bush-brown, *Bicyclus anynana*. The eyespot markings on the ventral side of their wings deter predators, while dorsal markings signal to potential mates; **(C)** Túngara frog, *Engystomops pustulosus.* Females prefer signals of higher complexity, which are also easier to detect by predators such as bats; and **(D)** Wolf spider *Hygrolycosa rubrofasciata*. Males vibrate their abdomen against the dry leaf substrate producing a drumming that is even audible for humans. Females prefer males with a high drumming rate, yet a high drumming rate can lead to increased predation risk. Photos: **(A)** PH Olsen CC BY 3.0, Wikimedia Commons; **(B)** Oskar Brattström; **(C)** R. Taylor; **(D)** Sanja565658 CC BY-SA 3.0, Wikimedia Commons.

the function and ecological significance of coloration and compounds used in chemical communication are well-known, to understand the interplay between sexual selection and selection by predators on the different components of multimodal signals. To this end, we use the wood tiger moth *A. plantaginis* as a case study; and (4) suggest specific paths for future research to test how aposematic signals can be used in mating contexts, and evolve under (the interacting effects of) natural and sexual selection.

APOSEMATISM IS INHERENTLY MULTIMODAL

Animal displays often consist of several components (Bradbury and Vehrencamp, 2011). When multiple components stimulate different sensory systems in the receiver, for example the visual and the auditory (**Figure 2A**), these displays are considered multimodal (Partan and Marler, 1999; Higham and Hebets, 2013). If these multiple components, however, elicit receiver responses in the same sensory modality, these displays are not considered multimodal and are referred to simply as multicomponent (Partan and Marler, 2005; Bradbury and Vehrencamp, 2011; Higham and Hebets, 2013). For example, a visual signal may contain several components such as color, pattern, and size (**Figure 2B**), which may even provide different information to the receiver, but in the end only stimulates one sensory (visual) modality (Rowe, 1999).

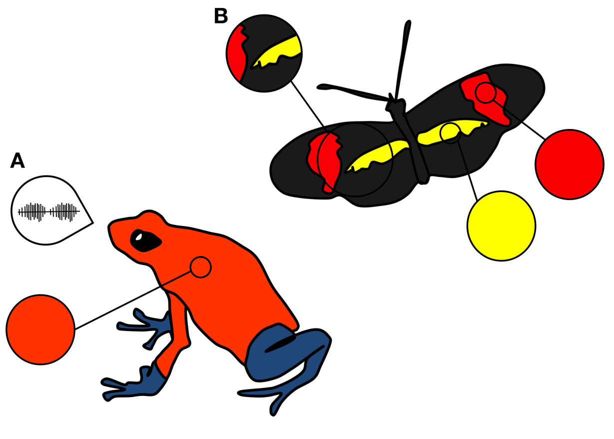

FIGURE 2 | Illustration of the difference between **(A)** multimodal and **(B)** multicomponent signals. **(A)** The strawberry poison frog, Oophaga pumilio, has both visual (color) and acoustic (call) signals. While both are involved in sexual selection (Maan and Cummings, 2012; Dreher and Pröhl, 2014), these stimulate different sensory (visual and auditory) channels or modes in the receiver. **(B)** The butterfly Heliconius erato has both color and pattern components to its visual signal (Finkbeiner et al., 2014). These may (or may not) encode different information, but stimulate the same sensory channel or mode (vision) in the receiver. Note that we are here focusing only on the visual signal of this butterfly for the purpose of illustrating multicomponency. H. erato is also chemically defended and the combination of its secondary defences and its visual signal make up their multimodal aposematic display.

Multimodal signals are thought to improve associative learning because they provide more information per unit of time than uni-modal displays (Partan and Marler, 2005) and, thus, the interaction between multiple types of signals is often expected to be more efficient than each signal on its own. However, there are various types of multimodal signals, which differ in the type of response they elicit in the receiver, depending on whether each component acts independently, exerts dominance or modulation over the other signal(s), or give rise to an entirely new response (Partan and Marler, 1999).

With the coupling of a warning signal and a secondary (e.g., chemical) defense, aposematic organisms are capable of deterring predators by stimulating, for example, their visual *and* olfactory/gustatory (chemical) sensory channels. Therefore, aposematism is inherently multimodal (Rowe and Guilford, 1999; Rowe and Halpin, 2013). The most common primary defense in warning displays is warning coloration (visual component). To ensure its efficacy as a signal, warning coloration is expected to be conspicuous and distinctive, and therefore easy to learn and memorize, as all these characteristics facilitate predator's associative learning (Cott, 1940). In fact, predators seem to remember the association between aposematic signals and unprofitability for longer than when learned for unprofitable cryptic species (Roper and Redston, 1987; Roper, 1994). Red, orange, and yellow have been suggested to be efficient warning signals given their color constancy under varied light environments, and their high contrast against different backgrounds (Stevens and Ruxton, 2012; **Figure 3**). Likewise, color patterns with high internal contrast, such as black and white or black and yellow, have been proven to be learned faster (Zylinski and Osorio, 2013).

In addition to visual signals, sounds such as the buzz of bumblebees (Siddall and Marples, 2011) or the ultrasonic clicks of some tiger moth species (Dunning and Kruger, 1995; Hristov and Conner, 2005; Ratcliffe and Nydam, 2008) have shown to protect defended prey from predators such as birds and bats, respectively. Likewise, skunks use warning sounds and behaviors to advertise the possession of chemical defenses, which they only spray if absolutely necessary (Andersen et al., 1982; Lartviere and Messier, 1996). Interestingly, although not conducted with the purpose of studying warning displays, a study by Tuttle and Ryan (1981) showed that the frog-eating bat, *Trachops cirrhosus*, is capable of distinguishing edible from unpalatable frogs on the basis of their mating calls (Tuttle and Ryan, 1981), hinting at a prominent role of warning signals of different sensory modalities in the deterrence of non-visually-oriented predators.

Among secondary defenses, the most prominent are defensive chemicals. Examples of chemical defenses in vertebrates include the alkaloids found in poison frogs (Saporito et al., 2012; Santos et al., 2016), the tetrodotoxins found in some newts, pufferfish and some harlequin toads (Mosher et al., 1964; Kim et al., 1975), and the disulfides (among other compounds) sprayed by skunks (Andersen et al., 1982). Among invertebrates, some common defensive compounds are the iridoid glycosides (Lindstedt et al., 2010; Reudler et al., 2015), cardenolides, pyrrolizidine alkaloids, pyrazines, and cyanide compounds found in numerous insects (Rothschild et al., 1979, 1984; Bowers, 1992), as well as the

FIGURE 3 | Aposematism is widespread across the animal kingdom. **(A)** Harlequin bug, *Tectocoris diophthalmus*; **(B)** Eastern newt (eft form), *Notophtalmus viridescens*; **(C)** Burnet moths, Family Zygaenidae; **(D)** Sea slug, *Chomodoris annae*; **(E)** Eastern coral snake, *Micrurus fluvius*; **(F)** Appalachian mountains millipede, *Apheloria polychroma*; **(G)** Harlequin poison frog, *Oophaga occultator*; **(H)** ladybird, family Coccinelidae. Photos: **(A)** E. Burdfield-Steel; **(B,D)** JP Lawrence; **(C,H)** B. Rojas; **(E)** N. Scobel; **(F)** P. Marek; **(G)** P. Palacios.

furanosesquiterpenes and diterpenes (among others) found in nudibranch molluscs (Winters et al., 2018). These defenses may stimulate the olfactory or gustatory channels, or both.

THE INTERPLAY BETWEEN NATURAL AND SEXUAL SELECTION IN SHAPING MULTIMODAL APOSEMATIC SIGNALS

Although there is no consensus about how aposematic coloration initially evolved, it has been suggested that it may have appeared as a co-option to some form(s) of intraspecific communication (**Figure 4**). That is, for example, markings allowing individual recognition (**Figure 4B**), or sexually selected traits being modified to have a double function (to ward-off would-be predators and either indicate status, or attract potential mates) once the species had developed an effective secondary defense (Mallet and Singer, 1987). Given our focus on aposematism we will primarily discuss natural selection imposed by predation pressure for the remainder of this review.

The evolution of warning signals via natural selection may be coupled with sexual selection in both a stabilizing or diverging manner, and both forces can work together on different temporal (e.g., juvenile vs. adult life stages) or spatial

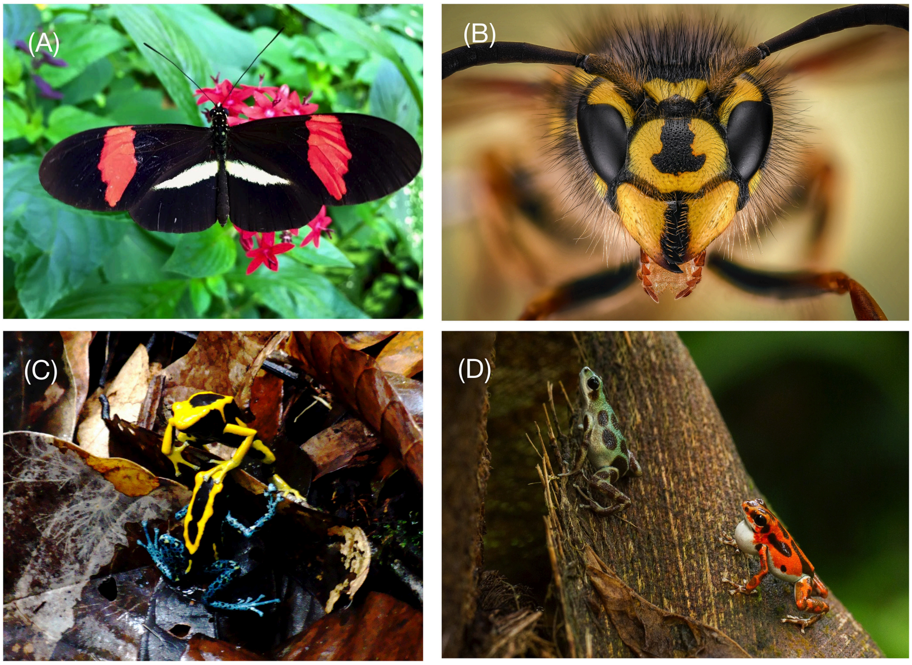

FIGURE 4 | In some aposematic species warning signals are also used for intraspecific communication. **(A)** In the postman butterfly, *Heliconius erato*, wing color pattern can serve a dual purpose in predator deterrence and mate attraction; **(B)** Some wasps can use their facial yellow-and-black markings for individual recognition and status signaling; **(C)** Males of the dyeing poison frog (*Dendrobates tinctorius*) tend to have yellower dorsal areas, possibly to enhance protection from predators during tadpole transport, while females with higher amounts of yellow in their frontal area are more often found in courtship; **(D)** In some populations of the strawberry poison frog (*Oophaga pumilio*), brighter males are preferred by females and also have a higher status in agonistic encounters with other males. Photos: **(A)** S. Finkbeiner; **(B)** E. Florin Niga; **(C)** B. Rojas; **(D)** A. Pašukonis.

(e.g., geographic) scales. Depending on the cognitive abilities of receivers (predators and mates) signals may be perceived differently, leading to differential selection and reaching different balances between them (Endler, 1992). An example of both forces acting at the same time can be observed in the strawberry poison frog, *Oophaga pumilio*, where females have been shown to prefer males with the brightest warning signal (Maan and Cummings, 2008) who, in turn, have the most noxious chemical defense (Maan and Cummings, 2012). This type of "honest signaling" of prey defenses may facilitate synergistic selection of both warning and sexual signal efficiency (Maan and Cummings, 2012; see details below) as females may benefit from mating with well-defended males.

When these dual selection pressures work in a divergent manner, sometimes they can cancel each other's effect or lead to fluctuating evolutionary responses of the warning signal depending on the selection strength of each side over time. For example, although under stabilizing selection by predators, female preference has been shown to facilitate phenotypic divergence through hybridization in harlequin poison frogs, *Oophaga histrionica* (Medina et al., 2013). In Neotropical longwing butterflies, genus *Heliconius*, two sister species (*H. melpomene* and *H. cydno*) have recently diverged to mimic different model taxa, which increases the survival benefits of both, but their mimetic coloration could lead to a cost associated to mate recognition in both species due to the time and energy spent while approaching and courting females of the co-mimic species (Jiggins et al., 2001; Estrada and Jiggins, 2008). Therefore, the multimodal nature of animal signals is prone to the evolution of complex biological interactions (Maynard Smith and Harper, 2003), yet these are seldom addressed simultaneously. In the following sections, we discuss in detail how natural and sexual selection can influence aposematic displays.

The Interactive Effect of Natural and Sexual Selection Can Maintain Intra-and Inter-Population Variation in Warning Coloration

The interplay between natural and sexual selection in the evolution of aposematic signals is particularly interesting in species in which the variability of the signal challenges the "uniformity" assumption of aposematism. Whilst a non-variable signal within a population is expected in order to favor predator avoidance learning (Endler, 1988a; Joron and Mallet, 1998; Mallet and Joron, 1999; Lindström et al., 2001; Endler and Mappes, 2004; Darst et al., 2006; Mallet, 2010; Chouteau et al., 2016), as stated above, a variable signal–without losing its conspicuous nature-could be associated with the relative attractiveness of some individuals over others (Ueno et al., 1998; Maan and Cummings, 2009). For that reason, aposematic species with a high *within*-population phenotypic variability are excellent models to test how both natural and sexual selection affect the evolution and design of warning signals.

Among the extensive scientific literature regarding aposematic species, only a handful of species have been studied in terms of how warning signal diversity varies intraspecifically both within and between populations. Whilst many studies have expanded our knowledge on the shape and function of warning signals, most have focused only on the emitter end (i.e., the prey). However, to understand the complexity of warning signal variation within and between populations, it is necessary to determine what are the pressures that could be affecting the survival and reproductive success in populations, and how these warning displays act in concert to outweigh the cost of their expression (Hebets and Papaj, 2005; Gohli and Hogstedt, 2009).

One of the species in which multiple selective factors have been studied in relation to warning signal evolution, within and between populations, is the strawberry poison frog, *Oophaga pumilio*. Several studies have shown that the geographic variation and polymorphism in its aposematic signals is the result of the combined action of natural and sexual selection. Predators avoid warningly colored plasticine models in the field (e.g., Saporito et al., 2007; Hegna et al., 2011), and controlled experiments in the laboratory have shown that not only do females prefer to mate assortatively with males of their own morph [Summers et al., 1999; Reynolds and Fitzpatrick, 2007; Maan and Cummings, 2008; but see Yang et al. (2016) for a study showing that assortative mating occurs in allopatric populations but not in sympatric ones], but also prefer overall males with brighter coloration (Maan and Cummings, 2009). However, calls (acoustic signals) seem to be more important than coloration for female choice (Dreher and Pröhl, 2014). Males hold territories that are defended from other males through calls, and calling activity and perch height, a proxy for exposure, are correlated with mating success (Pröhl and Hödl, 1999). Only the most conspicuous males can afford to use the more exposed calling sites (Rudh et al., 2011), as they are presumably better protected from predators. More conspicuous or brighter males are also bolder (Rudh et al., 2013) and more aggressive (Crothers et al., 2011; Crothers and Cummings, 2015), suggesting that aposematic signals in this species have also been co-opted as an indicator of fighting abilities (Crothers and Cummings, 2015).

In the dyeing poison frog, *Dendrobates tinctorius*, an interplay between natural and sexual selection affecting warning signals has also been proposed, although in lesser detail. Field studies with frog models at Nouragues Reserve (French Guiana) have shown that the warning signals of *D. tinctorius* elicit few avian predator attacks (Noonan and Comeault, 2009; Rojas et al., 2014) and are subject, as expected, to positive frequency-dependent selection (Comeault and Noonan, 2011). Males of this population have a higher proportion of yellow in their dorsal area than females, and the authors suggest that a synergy between sexual selection (in the form of parental care) and aposematism could select for yellower males (Rojas and Endler, 2013). Females, in contrast, seem to be favored by sexual selection when they present a higher amount of yellow in their frontal area, which is highly visible during courtship interactions (Rojas, 2012). Pairs in this population show no signs of assortative mating for color patterns, which could help explain the high phenotypic variation observed (Rojas, 2012).

As with vertebrates, studies aiming to explain warning color polymorphism within populations of arthropods are mainly focused on frequency-dependent selection (Benson, 1972; Mallet and Barton, 1989; Langham, 2004; Borer et al., 2010; Nokelainen et al., 2014) or mating preferences (Chouteau et al., 2016). Unlike vertebrates, however, insects have been well-studied at different life stages in relation to aposematic signals and their interplay with allelochemical sequestration (Marples et al., 1994; Roque-Albelo et al., 2002), allowing the opportunity to study carry-over effects of early life on the adult expression of warning coloration and chemical defenses.

Multiples studies have focused on the striking warning signal polymorphism observed in some ladybeetles (family Coccinnelidae). For instance, Osawa and Nishida (1992) and Awad et al. (2015) showed that polymorphism in the elytra coloration of *Harmonia axyridis* is maintained either by seasonal mating variation or assortative mating (respectively; Osawa and Nishida, 1992; Awad et al., 2015). In *Adalia bipunctata*, in contrast, the polymorphism is maintained by assortative mating coupled with inheritance of female preference (Majerus et al., 1982), showing negative frequency-dependent mating selection (i.e., females prefer the rare morph; O'Donald and Majerus, 1984).

Another group in which these two selective pressures have been widely studied is the Neotropical butterflies of the genus *Heliconius*. These butterflies, which occur in Central and Northern South America, are characterized by wings with conspicuous markings (e.g., yellow, white, red, etc.) on a dark background, which inform predators about the possession of cyanide compounds that make them toxic (Nahrstedt and Davis, 1983; Zagrobelny et al., 2004; Cardoso and Gilbert, 2013). In this genus, the evolution of distinct color patterns between populations has been extensively explained in the context of Müllerian mimicry, in which a warningly colored aposematic species mimics the appearance of another one to share the costs of predator education (Müller, 1879). Although both the composition and spatial variation of the predator communities selecting for this resemblance in their coloration are still unknown (Merrill et al., 2015), these mimetic species have become a textbook example of natural selection (Jiggins, 2017). However, others studies have also explored how sexual selection, via mate choice and assortative mating (Jiggins et al., 2001; Estrada and Jiggins, 2008; Merrill Richard et al., 2014), has shaped wing coloration. For example, in a recent study, Finkbeiner et al. (2014) tested the relative importance of color and pattern in predation avoidance and mate choice in *Heliconius erato*. The authors found that although the right combination of local color and pattern provided the highest deterrence and mate attraction, color seemed to be more important than pattern, suggesting that sexual and natural selection work in parallel to influence the evolution of warning coloration in this species (Finkbeiner et al., 2014).

Interactive Effects of Natural and Sexual Selection May Lead to Population Divergence and Speciation

Inter-population variation in multimodal warning signals provides an opportunity for unraveling how populations diverge and, eventually, in some cases, how new species originate. This can also occur through the joint effect of natural and sexual selection on aposematic traits (Maan and Seehausen, 2011). Population divergence through natural selection alone would require extreme combinations of parameters (e.g., almost null migration and strong selection for ecological specialization) to be fulfilled because gene flow would decrease the level of diversification (Mayr, 1963). Therefore, the effects of sexual selection are required to promote sexual isolation (through pre- and post-zygotic mechanisms), together with the effect of linkage disequilibrium to maintain the traits correlated and inherited by the following generation (Servedio, 2009). This interplay is particularly important for ecological speciation in sympatry, which occurs when reproductive isolation has evolved as an adaptation to different environments (reviewed in Rice and Hostert, 2017), or through hybridization, which can generate novel traits capitalizing on existing variation between related species (Mallet, 2007; Salazar et al., 2010). Additionally, the relaxation of predation pressure on aposematic species leaves room for traits to be selected by sexual selection, especially if predators associate these mating signals with unprofitability.

As seen in the previous section, poison frogs (family Dendrobatidae) can use warning coloration as a mating signal. However, the predominant modality of anuran mating signals is acoustic (i.e., advertisement calls). A recent study by Santos et al. (2014) demonstrated that acoustic mating signals in poison frogs (Dendrobatidae) have diversified in association with aposematism due to sexual selection, such that aposematic species have calls with a set of characteristics that differ from those of non-aposematic species (Santos et al., 2014). The level of conspicuousness in different populations of *O. pumilio* also predicts other aspects of the sexual display behavior, with males from more conspicuous populations calling from more exposed sites (Pröhl and Ostrowski, 2011; Rudh et al., 2011), and being more aggressive and explorative (Rudh et al., 2013). These behavioral differences coupled with mechanisms such as assortative mating could generate pre-zygotic isolation leading to population divergence, in the first place, and potentially to a speciation process in the long term. Indeed, in *O. pumilio*, molecular approaches show that color, but not body size, is diverging at high rates, indicating selection (Wang and Shaffer, 2008; Brown et al., 2010). Moreover, these studies demonstrate that sexual and natural selection are causing genetic isolation between different color morphs in the wild, which could be a sign of incipient speciation (Wang and Summers, 2010). This is supported by recent findings showing that, within Dendrobatidae, the aposematic lineages are speciating at higher rates than their non-aposematic counterparts (Santos et al., 2014).

The synergistic effects of sexual selection and natural selection are also likely to affect speciation processes in *Heliconius* butterflies. The color and pattern of their wings (reviewed in Jiggins, 2017), coupled to a very characteristic flight behavior (Srygley, 1999), help predators recognize and subsequently avoid them, but the former are also involved in mate recognition. This suggests that the ultimate fitness of individuals displaying different combinations of these traits is determined by both synergies and compromises between the different functions (Merrill et al., 2015). Several of these species belong to local mimicry rings, making their appearance the subject of strong purifying selection, but also strong assortative mating (Jiggins et al., 2001). Under these conditions, novel forms are punished by a higher predation due to frequency-dependent selection (Mallet and Barton, 1989). Hybrids would be expected to have the same fate if their appearance deviates from the parental phenotype(s) (Merrill et al., 2012); however, one of the most fascinating aspects of this system is that hybridization has offered a route to speciation (Mavárez et al., 2006; Mallet, 2007; Salazar et al., 2010). Wing color patterns in *Heliconius* are thus involved in predator deterrence, species recognition, and mating preferences. However, colors can be only one component of a multimodal mating signal that also involve chemical components, e.g., pheromones. Even in a community consisting of mimetic species, visual attraction can be based at first on wing appearance, yet at a shorter range scents from the wings and the genitalia can provide species-specific chemical signatures leading to assortative mating (Mérot et al., 2015).

Chemical Compounds Can Play a Role in Mate Attraction and Predator Deterrence—But Could They Also Have a Dual Function?

Insects offer a prime example of sexual communication mediated by chemical signals such as pheromones. As such, the divergence in pheromone components has shown to play a key role also in speciation (Groot et al., 2006, 2009). Pheromone composition and variability have been studied in detail in bella moths, *Utheteisa ornatrix* (Conner et al., 1981), moths in the genus *Heliotis* (Klun et al., 1980; Teal et al., 1984; Heath et al., 1991) and bark beetles (genus *Ips*; Lanier et al., 1980; Seybold et al., 1995). As well as long-range pheromones, cuticular hydrocarbons (or CHCs) have also been shown to play an important role in intraspecific communication and mate choice in insects (Sharma Manmohan et al., 2011; Ingleby, 2015).

The use of defensive chemicals is also widespread throughout insects. In addition to their crucial role in predator deterrence, a linkage between defensive chemicals and intraspecific communication has been shown in many insect species. For instance, in Lepidoptera, different families (e.g., Nymphalidae, Danaidae, and Erebidae) use secondary compounds such as pyrrolizidine alkaloids in male courtship displays, nuptial gifts, and egg protection (Boppré et al., 1978; Brown, 1984; Moore et al., 1990; Weller et al., 1999). For example, males of *U. ornatrix*, have glandular structures in which they store pyrrolizines. Males unable to produce certain compound derived from these alkaloids have been found to be less successful at courting females (Conner et al., 1981). In fact, it has been suggested that this compound is used by females to assess the extent to which the male is chemically protected (Conner et al., 1981). A similar example can be found in the beetle *Neopyrochroa flabellate* (Eisner et al., 1996a,b).

However, we currently have very little information on whether sexual selection and natural selection shape the secondary defenses synergistically. In some aposematic species, levels of secondary defense have shown to differ between females and males at the reproductive life-stage, which may suggest differential selection. For example, burying beetles (*Nicrophorus vespilloides*) use their anal exudates both for their own defense and the protection of their offspring, and females appear to produce more of these exudates than males (Lindstedt et al., 2017). Allocation for chemical defense has also shown to trade off with reproductive success indicating that these two functions could play important role in both mate attraction and predator deterrence (Nokelainen et al., 2012). This interaction is further complicated by the fact that many species sequester their chemical defenses from their diet. In the true bug *Lygaeus equestris*, for example, diet, and therefore level of chemical defense, has no effect on mate choice (Burdfield-Steel et al., 2013). This is despite evidence that females of this species pass defensive chemicals on to their eggs, protecting them from predators (Newcombe et al., 2013). When variation between individuals is purely environmental, effects on mate choice may only occur when direct benefits are high (as in several of the examples given above), although see (Geiselhardt et al., 2012) for an example of diet and host plant leading to associative mating based on CHC (cuticular hydrocarbons) profile. When direct benefits are low, species in which chemical defense level is either genetically determined, or indicative of overall quality, may be better candidates in which to look for mate choice based on defense level. This may well be the case in species that produce their defenses *de novo*.

MOVING TOWARD A MORE INTEGRATED VIEW OF APOSEMATIC SIGNALS

Despite all the examples discussed so far, it is clear that only a few studies address how both natural and sexual selection act (either synergistically or antagonistically) on the evolution of multimodal aposematic signals. Furthermore, it is apparent that not only color, but also odor, taste, and behavior are part of warning displays, and their interaction, besides strengthening the signal, can provide reliable information about the quality of the emitter. Yet, only a handful of studies have considered the interplay among these, and their joint significance remains barely tested. To corroborate these impressions, we conducted a literature search in Web of Science and analyzed the contents of a representative sample of the articles available on aposematism. We used the search terms "aposematism or aposematic" to have the widest spectrum possible of studies and organisms, and limited the search to articles published in or after 1990 and until mid-April 2018. This search rendered a total of 1,051 articles, out of which we analyzed 105 (10%) selected as explained below.

Because taking the first (or last) articles in the search list would have constrained the timeframe, we took the first 10% of the number of articles published each year, which varied between 10 in 1990 and 87 in 2017 (**Figure 5**), that fitted the following criteria. We only included articles studying an actual natural animal system (i.e., no plants), and assessing directly or indirectly the effect of natural (predation) and/or sexual selection in the signals considered. Therefore, studies done with artificial prey represented as symbols or using artificial chemicals were excluded. Artificial prey were accepted if they aimed to represent the actual animal studied, as in dummies or models. We also excluded taxonomic descriptions, as well as phylogenetic and phylogeographic studies in which there was no direct relation with the selection pressures on which we focus this review. For each paper we recorded the focal species identity, the trait(s) studied, whether or not they consider the multimodality aspect, and the type of selection addressed (**Table 1**).

As revealed by our search, aposematism is a phenomenon that has raised increasing interest among researchers over the last three decades (**Figure 5**), and has been studied in a variety of organisms (**Figure 3**), spanning gastropods through to carnivores. Nevertheless, invertebrates seem to be studied more, in ∼69% of the cases (**Figure 6A**); perhaps not surprising considering they cover 97% of organisms on earth. Both within invertebrates and vertebrates, there are taxonomic groups accounting for the majority of the studies (**Figure 6A**). Among invertebrates, the best studied are lepidopterans (34.2%), such as longwings (5.7%), coleopterans (20.5%), and other insects (34.2%). Among vertebrates, poison frogs are undoubtedly the group that has stimulated most research (68.9%), followed by snakes (18.2%; **Figure 6A**).

Regardless of the taxonomic group, most studies have focused on unimodal signals, particularly visual (59.4%), and chemical (16%; **Figure 6B**). Multimodal signals were studied only in 17.9% of the cases, and consisted in all cases of visual signals in

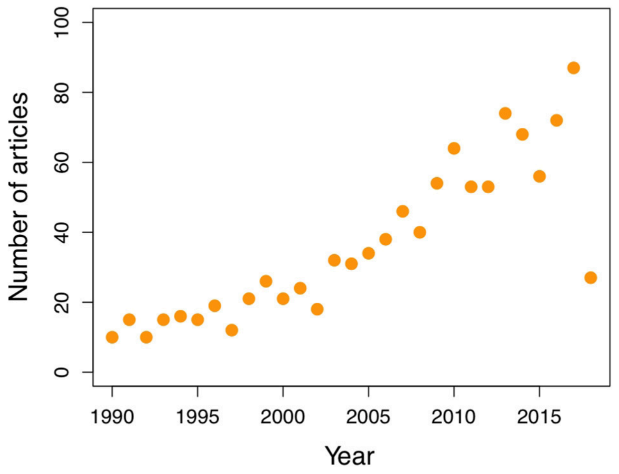

FIGURE 5 | The number of studies on aposematism has been increasing over the last three decades. See text for details on data used.

TABLE 1 | Studies included in the review of literature published on aposematism over the last three decades.

Year	Organism	Common name	Higher classification	Selection pressur addressed	Trait(s) studied	References
2018	*Pseudophryne spp*	Australian brood frogs	Anura	pred	col	Lawrence et al.
2018	*Nudibranchs*	Sea slug	Gastropoda	pred	chemdef	Winters et al.
2018	*Andinobates bombetes*	Poison frog	Anura	pred	col	Casas-Cardona et al.
2017	*Vipera seoanai*	Iberian cross adder	Squamata	pred	col+patt	Martínez-Freiria et al.
2017	*Nicrophorus vespilloides*	Burying beetle	Coleoptera	both	col+chemdef	Lindstedt et al.
2017	*Oophaga pumilio*	Strawberry poison frog	Anura	pred	col+patt	Preissler and Pröhl
2017	*Arctia plantaginis*	Wood tiger moth	Lepidoptera	pred	chemdef	Rojas et al.
2017	*Danainae*	Milkweed butterfly	Lepidoptera	pred	col	Aluthwattha et al.
2017	*Pyrrhocoris apterus*	Firebug	Hemiptera	pred	col	Landova et al.
2017	*Nudibranchs*	Sea slug	Gastropoda	pred	col+chemdef	Winters et al.
2017	*Pyrrhocoris apterus*	Firebug	Hemiptera	pred	col	Benes and Vesely
2017	*Heliconius*	Longwings, heliconians	Lepidoptera	both	col+patt	Chouteau et al.
2016	*Heliconius*	Longwings, heliconians	Lepidoptera	pred	chemdef	Arias et al.
2016	*Bombus*	Bumblebee	Hymenoptera	pred	sound	Moore and Hassle
2016	*Arctia plantaginis*	Tiger moth	Lepidoptera	both	col	Lindstedt et al.
2016	*Oophaga pumilio*	Strawberry poison frog	Anura	ss	col	Gade Et al.
2016	*Heliconius*	Longwings, heliconians	Lepidoptera	pred	col	Dell'Aglio et al.
2016	*Pyrrhocoris apterus*	Firebug	Hemiptera	pred	col	Adamova-Jezova et al.
2016	*Lampyridae*	Firefly	Coleoptera	pred	chemdef	Vencl et al.
2015	*Indian butterflies*	Other butterfly	Lepidoptera	both	col	Su Et al.
2015	*Arctia plantaginis*	Wood tiger moth	Lepidoptera	pred	pattern	Honma et al.
2015	*Arctia plantaginis*	Wood tiger moth	Lepidoptera	both	col	Gordon et al.
2015	*Adalia bipunctata*	Two-spotted ladybird	Coleoptera	both	chemdef	Paul et al.
2015	*Pyrrhocoris apterus*	Firebug	Hemiptera	pred	col	Exnerova et al.
2015	*Dendrobates inctorius*	Dyeing poison frog	Anura	pred	col+patt	Hämäläinen et al.
2014	*Euphydryas and Chlosyne*	Other butterfly	Lepidoptera	pred	col+chemdef	Long et al.
2014	*Heliconius erato*	Longwings, heliconians	Lepidoptera	both	col+patt	Finkbeiner et al.
2014	*Paederus fuscipes*	Rove beetle	Coleoptera	pred	chemdef	Tabadkani and Nozari
2014	*Dendrobates tinctorius*	Dyeing poison frog	Anura	pred	col+patt	Rojas et al.
2014	*Arctia plantaginis*	Wood tiger moth	Lepidoptera	pred	pattern	Hegna and Mappes
2014	*Oophaga pumilio*	Strawberry poison frog	Anura	pred	col+patt	Qvarnström et al.
2014	*Oophaga granulifera*	Strawberry poison frog	Anura	pred	col	Willink et al.
2013	*Rana rugosa*	Japanese wrinkled frog	Anura	pred	chemdef	Yoshimura and Kasuya
2013	*Oophaga histrionica*	Harlequin poison frog	Anura	ss	col	Medina et al.
2013	*Oophaga granulifera*	granular poison frog	Anura	pred	col	Willink et al.
2013	Heteroptera	True bugs	Hemiptera	pred	col	Svadova et al.
2013	*Oophaga pumilio, Oophaga granulifera*	Strawberry poison frog, granular poison frog	Anura	both	col+sound	Pröhl et al.
2013	*Flabellina iodinea*	Sea slug	Gastropoda	pred	chemdef	Noboa and Gillette
2013	*Oophaga pumilio*	Strawberry poison frog	Anura	pred	col	Hegna et al.
2012	*Heliconius*	Longwings, heliconians	Lepidoptera	pred	col+patt	Merrill Et al.
2012	*Oophaga pumilio*	Strawberry poison frog	Anura	pred	col	Stuart et al.
2012	*Polistes dominula*	European paper wasp	Hymenoptera	pred	col+chemdef	Vidal-Cordero et al.

(Continued)

TABLE 1 | Continued

Year	Organism	Common name	Higher classification	Selection pressur addressed	Trait(s) studied	References
2012	*Vipera spp.*	European vipers	Squamata	pred	col+patt	Valkonen et al.
2012	True bugs	True bugs	Hemiptera	pred	chemdef	Noge et al.
2011	*Ranitomeya imitator*	Mimic poison frog	Anura	pred	col	Chouteau and Angers
2011	*Lycorma delicatula*	Spotted lanternfly	Hemiptera	pred	col	Kang et al.
2011	*Motyxia spp*	Millipede	Myriapoda	pred	luminescence	Marek et al.
2011	*Oophaga pumilio*	Strawberry poison frog	Anura	both	col	Ozel and Stynoski
2011	*Cynops pyrrhogaster*	Japanese fire belly newt	Caudata	pred	col	Mochida
2010	*Oreina gloriosa*	Leaf beetles	Coleoptera	pred	col	Borer et al.
2010	*Graphosoma lineatum*	Shield bugs	Hemiptera	pred	col	Johansen et al.
2010	*Bombus spp*	Bumblebee	Hymenoptera	pred	col	Stelzer et al.
2010	*Pyrrhocoris apterus*	Firebug	Hemiptera	pred	col+size	Prokopova et al.
2010	*Opistobranchs*	Sea slug	Gastropoda	pred	col+chemdef	Cortesi and Cheney
2010	*Hypselodoris fontandraui*	Sea slug	Gastropoda	pred	col+chemdef	Haber et al.
2009	*Oophaga pumilio*	Strawberry poison frog	Anura	ss	col	Maan and Cummings
2009	*Mephitis mephitis*	Skunk	Carnivora	pred	col+shape	Hunter
2009	*Photinus*	Firefly	Coleoptera	pred	luminescence	Moosman et al.
2009	Ladybirds	Ladybird	Coleoptera	pred	pattern+shape	Dolenska et al.
2009	Tiger moths	Tiger moth	Lepidoptera	pred	sound	Barber et al.
2008	Tiger moths	Tiger moth	Lepidoptera	pred	col+sound	Ratcliffe and Nydam
2008	*Oophaga pumilio*	Strawberry poison frog	Anura	ss	col	Maan and Cummings
2008	Carabid beetles	Ground beetle	Coleoptera	pred	col+chemdef	Bonacci et al.
2008	Lycidae beetles	Net-winged beetle	Coleoptera	pred	chemdef	Eisner et al.
2007	*Oophaga pumilio*	Strawberry poison frog	Anura	pred	col	Saporito et al.
2007	*Cirriformia punctata*	Polychaete	Polychaeta	pred	chemdef	Meredith et al.
2007	*Oophaga pumilio*	Strawberry poison frog	Anura	ss	col	Reynolds and Fitzpatrick
2007	*Harmonia axyridis*	Asian ladybeetle	Coleoptera	pred	col+chemdef	Bezzerides et al.
2007	Tiger moths	Tiger moth	Lepidoptera	pred	sound	Barber and Conner
2006	*Micrurus phyrrocryptus*	Coral snake	Squamata	pred	colpatt	Buasso et al.
2006	*Carabid beetles*	Ground beetle	Coleoptera	pred	col+chemdef	Bonacci et al.
2006	*Graphosoma lineatum*	Shield bugs	Hemiptera	pred	col+chemdef	Veseley et al.
2006	*Pyrrhocoris apterus*	Firebug	Hemiptera	pred	col	Exnerova et al.
2005	*Cycnia tenera*	Tiger moth	Lepidoptera	pred	sound	Ratcliffe and Fullard
2005	*Vipera berus*	Common European adder	Squamata	pred	pattern	Niskanen and Mappes
2005	*Ensatina eschscholtzii xanthoptica*	Lungless salamander	Caudata	pred	col	Kuchta
2004	*Vipera berus*	Common European adder	Squamata	pred	pattern	Wüster et al.
2004	*Heliconius*	Longwings, heliconians	Lepidoptera	pred	col+patt	Langham
2004	*Oophaga pumilio*	Strawberry poison frog	Anura	both	col	Siddiqi et al.
2003	Poison frogs	Poison frog	Anura	pred	col+size	Hagman and Forsman
2003	Poison frogs	Poison frog	Anura	pred	col+chemdef	Santos et al.

(Continued)

TABLE 1 | Continued

Year	Organism	Common name	Higher classification	Selection pressur addressed	Trait(s) studied	References
2003	*Vespula norwegica*	Norwegian wasp	Hymenoptera	pred	col+shape	Kauppinen and Mappes
2002	*Eumaeus minyas*	Lycaenid butterfly	Lepidoptera	pred	chemdef	Castillo-Guevara and Rico-Gray
2002	*Murgantia histrionica*	Harlequin bug	Hemiptera	pred	chemdef	Aliabadi et al.
2001	*Pseudoxycheila tasalis*	Neotropical tiger beetle	Coleoptera	pred	col	Schultz
2001	*Pseudoxycheila tasalis*	Neotropical tiger beetle	Coleoptera	pred	chemdef	Schultz and Puchalski
2000	Cercopidae	Froghopper	Hemiptera	pred	col+chemdef	Peck
2000	*Schistocerca gregaria*	Desert locust	Orthoptera	pred	col+chemdef	Sword et al.
1999	*Cosmopepla bimaculata*	Stink bug	Hemiptera	pred	chemdef	Krall et al.
1999	Nudibranchs	Sea slug	Gastropoda	pred	col	Giménez-Casalduero et al.
1999	*Bombus terrestris*	Buff-tailed bumblebee	Hymenoptera	pred	sound	Kirschner and Roschard
1998	*Romalea guttata*	Lubber grasshopper	Orthoptera	pred	col+behav	Hatle and Faragher
1998	Flatworms	Flatworms	Platyhelminthes	pred	col	Ang and Newman
1997	*Ithomiine* and tiger moths	Tiger moth	Lepidoptera	pred	chemdef	Cardoso
1996	Neotropical butterflies	Neotropical butterflies	Lepidoptera	pred	col+behav	Pinheiro
1996	*Mephitis mephitis*	Striped skunk	Carnivora	pred	sound+behav	Lartviere and Messier
1995	Tiger moths	Tiger moth	Lepidoptera	pred	sound	Dunning and Kruger
1995	Coral snakes	Coral snake	Squamata	pred	col+patt	Brodie and Janzen
1994	*Coccinella septempunctata*	Seven-spot ladybird	Coleoptera	pred	col+chemdef	Marples et al.
1994	*Opistobranchs*	Sea slug	Gastropoda	pred	col+chemdef	Tullrot
1993	*Catocala spp*	Underwing moths	Lepidoptera	pred	col	Ingalls
1993	*Monistria concinna*	Grasshopper	Orthoptera	pred	col+chemdef	Groeters and Strong
1992	Tiger moths	Tiger moth	Lepidoptera	pred	sound	Dunning et al.
1991	*Polycera quadrilineata*	Sea slug	Gastropoda	pred	col	Tullrot and Sundberg
1991	Leaf beetles	Leaf beetle	Coleoptera	pred	chemdef	Pasteels and Rowellrahier
1990	*Battus philenor*	Blue swallowtail	Lepidoptera	pred	col	Codella and Lederhouse

See main text for details on inclusion criteria. pred, predation; ss, sexual selection; col, color; patt, pattern; chemdef, chemical defenses.

References in chronological order (from oldest to newest): (Codella and Lederhouse, 1990; Pasteels and Rowellrahier, 1991; Tullrot and Sundberg, 1991; Dunning et al., 1992; Groeters and Strong, 1993; Ingalls, 1993; Marples et al., 1994; Tullrot, 1994; Brodie and Janzen, 1995; Dunning and Kruger, 1995, 1996; Lartviere and Messier, 1996; Pinheiro, 1996; Cardoso, 1997; Ang and Newman, 1998; Hatle and Faragher, 1998; Gimenez-Casalduero et al., 1999; Kirchner and Roschard, 1999; Krall et al., 1999; Peck, 2000; Sword et al., 2000; Schultz, 2001; Schultz and Puchalski, 2001; Aliabadi et al., 2002; Castillo-Guevara and Rico-Gray, 2002; Kauppinen and Mappes, 2003; Santos et al., 2003; Langham, 2004; Siddiqi et al., 2004; Wuster et al., 2004; Kuchta, 2005; Niskanen and Mappes, 2005; Bonacci et al., 2006, 2008; Buasso et al., 2006; Exnerová et al., 2006, 2015; Vesely et al., 2006; Barber and Conner, 2007; Bezzerides et al., 2007; Meredith et al., 2007; Reynolds and Fitzpatrick, 2007; Saporito et al., 2007; Eisner et al., 2008; Maan and Cummings, 2008, 2009; Ratcliffe and Nydam, 2008; Barber et al., 2009; Dolenska et al., 2009; Hunter, 2009; Moosman et al., 2009; Borer et al., 2010; Cortesi and Cheney, 2010; Haber et al., 2010; Johansen et al., 2010; Prokopova et al., 2010; Stelzer et al., 2010; Chouteau and Angers, 2011; Kang et al., 2011; Marek et al., 2011; Mochida, 2011; Ozel and Stynoski, 2011; Pröhl and Ostrowski, 2011; Merrill et al., 2012; Noge et al., 2012; Stuart et al., 2012; Valkonen et al., 2012; Vidal-Cordero et al., 2012; Hegna et al., 2013; Medina et al., 2013; Noboa and Gillette, 2013; Pröhl et al., 2013; Svadová et al., 2013; Willink et al., 2013, 2014; Yoshimura and Kasuya, 2013; Finkbeiner et al., 2014; Hegna and Mappes, 2014; Long et al., 2014; Qvarnström et al., 2014; Rojas et al., 2014, 2017; Tabadkani and Nozari, 2014; Gordon et al., 2015; Hämäläinen et al., 2015; Honma et al., 2015; Su et al., 2015; Adamova-Jezova et al., 2016; Arias et al., 2016; Dell'aglio et al., 2016; Gade et al., 2016; Lindstedt et al., 2016, 2017; Moore and Hassall, 2016; Vencl et al., 2016; Aluthwattha et al., 2017; Benes and Vesely, 2017; Chouteau et al., 2017; Landová et al., 2017; Martinez-Freiria et al., 2017; Preissler and Pröhl, 2017; Winters et al., 2017, 2018; Casas-Cardona et al., 2018; Lawrence et al., 2018).

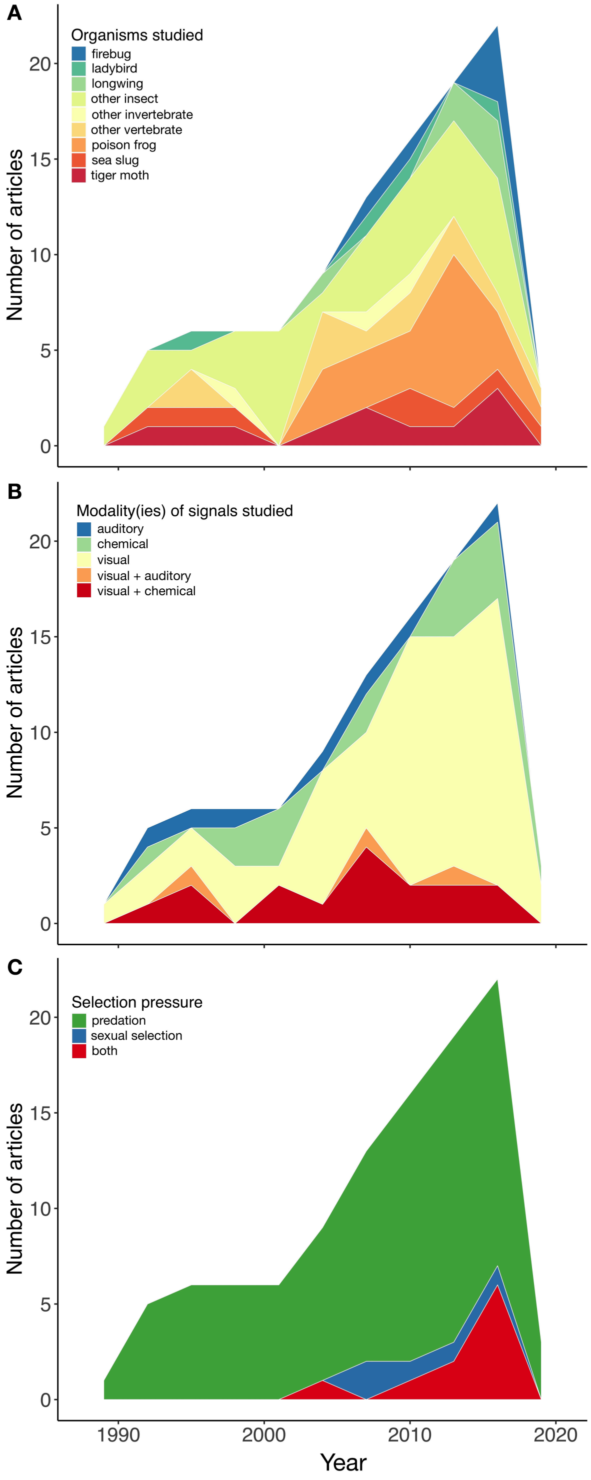

FIGURE 6 | (A) Representative animal groups studied in relation to aposematism over the last three decades. The larger the area, the more studies on that particular group. **(B)** Signaling modalities studied in relation to aposematism over the last three decades. The larger the area, the more studied. **(C)** Selection pressures taken into account in studies on aposematism over the last three decades. The larger the area, the more studies taking that particular selective pressure into account.

combination with either chemical defenses (15.1%) or auditory signals (2.8%; **Figure 6B**).

Not surprisingly, most studies on aposematism have addressed either directly or indirectly the effect of natural selection (predation) on the studied signals. As pointed out above, however, there is an increasing interest in systems or contexts in which the effect of both natural and sexual selection can be studied simultaneously. Of all the studies reviewed, only 4.7% addressed exclusively the effect of sexual selection on warning signals, while 9.4% investigated the effects of both selective forces jointly (**Figure 6C**). Most importantly, studies in which the effects of natural and sexual selection are investigated at the same time tend to focus only on one sensory modality, in particular the visual, even if two components of a signal, for example color and pattern, are taken into account. This tendency seems to be as true for vertebrates, as it is for invertebrates (**Figure 7**).

Studies addressing the influence of predation on multimodal signals seem to be slightly more common in invertebrates than in vertebrates (**Figure 7**). This is most likely because insects, the invertebrate group most studied in this context, can be more easily bred and kept in the laboratory due to their short-generation times and numerous offspring, and studied under manipulated conditions. Moreover, in many cases it is easier to disentangle the visual and chemical components of their multimodal warning displays (Marples et al., 1994; Rönkä et al., 2018a,b). Most importantly, the overrepresentation of some groups in these studies may be partially due to the dynamics of predator-prey coevolution and the speed to respond to selection (Härlin and Härlin, 2003). These may favor aposematism in organisms such as insects, amphibians, or reptiles, which lean toward an r-strategy (numerous offspring, high growth rate and low per capita probability of survival), while constraining it in organisms such as mammals and birds, which lean toward a K-strategy (few offspring, low growth rate and high per capita probability of survival), and are more often the selective agents.

To investigate this further, not only do we need new model species with well-studied visual signals and chemical communication, but also where the traits in question are heritable, and known to be under identified selective pressures (i.e., predation and sexual selection). Here, we propose the wood tiger moth, *A. plantaginis,* as one of such emerging model species where multimodal warning displays can be studied while addressing conflictive or synergistic selective pressures, as stated below.

THE WOOD TIGER MOTH AS A PROMISING MODEL TO STUDY MULTIMODALITY IN APOSEMATIC AND SEXUAL SIGNALING

One of the species in which multimodal aposematic signals have been studied in depth is *A. plantaginis* (formerly *Parasemia plantaginis*; Rönkä et al., 2016), the wood tiger moth (**Figure 8**). *A. plantaginis* is an aposematic diurnal moth with a widespread

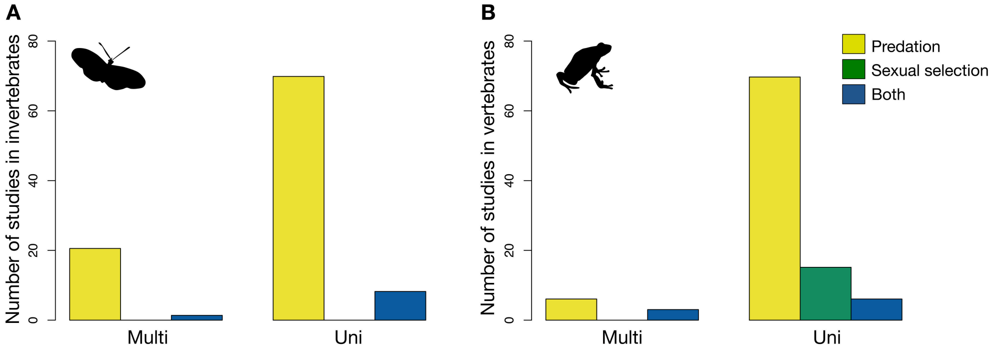

FIGURE 7 | Number of studies that address the effects of predation, sexual selection, or both on one (unimodal) or two (multimodal) signals in the aposematic displays of **(A)** invertebrates, and **(B)** vertebrates.

FIGURE 8 | The different morphs of the wood tiger moth in Europe. The upper row shows polymorphic color variation in males, whereas the bottom row showcases the continuous variation observed in females. Reproduced with permission from Nokelainen (2013). Photos: S. Waldron.

geographic distribution across the holarctic region (Hegna et al., 2015). Larvae of this species are polyphagous, feeding on a large number of different genera (Ojala et al., 2005), and overwinter at their 4th—5th instar. The adult stage lasts for about 2–3 weeks during which these moths do not feed. This means that both their coloration and chemical defenses are set at the larval stage (Ojala et al., 2005). Males spend their adult life flying in search of females, who are ready to mate soon after eclosion.

While their coloration has been shown to have a strong hereditary component (Nokelainen et al., 2013; Lindstedt et al., 2016), diet can also influence adult coloration, particularly in females (Lindstedt et al., 2010; Furlanetto, 2017; **Figure 9**). In addition, their chemical defenses are affected by resource availability during early life (Brain, 2016; Furlanetto, 2017; **Figure 9**). As the moths are most active during daylight hours (Rojas et al., 2015a) they are vulnerable to predation by birds, particularly while resting on the vegetation, where they are clearly conspicuous (Nokelainen et al., 2012; Henze et al., 2018). Likewise, they can be vulnerable to attacks by invertebrate predators, especially when the temperature is not high enough for the moth to initiate flight, or when it is eclosing from the pupa and its wings are not yet fully extended. Adult moths defend themselves with two distinct defensive fluids, one produced from the anal tract and one from glands behind the head (**Figure 9**). The first is targeted toward invertebrate predators, while the second is targeted toward avian predators (Rojas et al., 2017) and contains pyrazines, which the moths produce *de novo* (Burdfield-Steel et al., 2018), likely on the basis of constituents obtained from their diet. These defenses are advertised to birds with brightly colored hindwings, where red, yellow, or even white coloration is contrasted with black patterning (**Figure 8**).

Predation Is a Strong Selective Pressure on Wood Tiger Moth Warning Coloration and Chemical Defenses

Surprisingly, given their role in predator deterrence, the hindwings of wood tiger moths show considerable color variation, both within and between populations (Hegna et al., 2015; **Figure 8**). In the Finnish population, which has been the focus of much of the research on this species, males show discrete color polymorphism, possessing either white or yellow hindwings, while females vary continuously from yellow to red. In contrast, in the putative ancestral populations (Caucasus) males exhibit continuous variation from yellow-orange through to red in their hindwing coloration, while females display red hindwings. The forewings, on the other hand, do not vary much within populations, and consist of high-contrast black and white patterning. Many studies to date have demonstrated the predator-deterrent nature of this moth's coloration (Lindstedt et al., 2011;

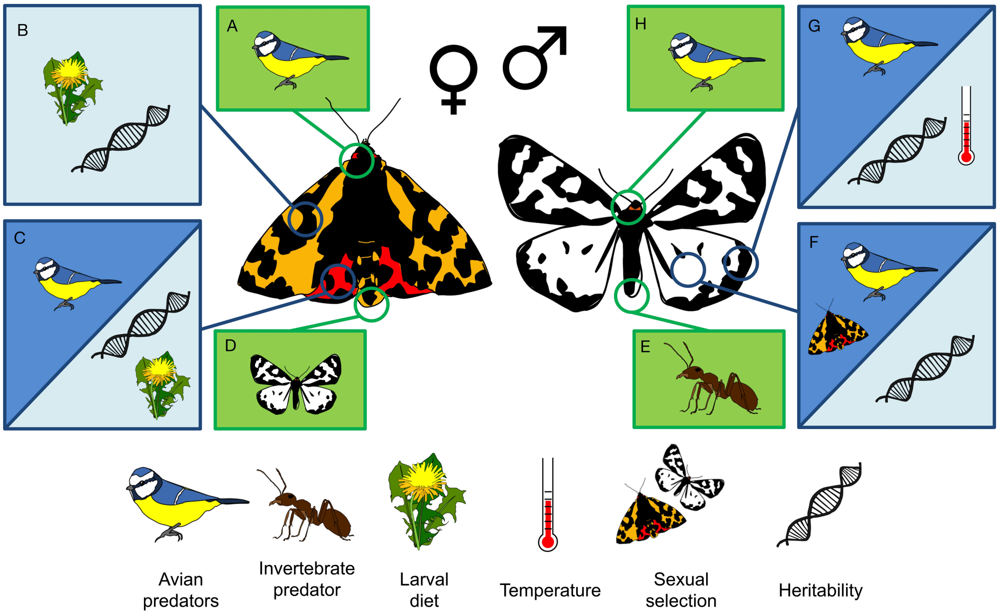

FIGURE 9 | A schematic illustration of the different selective (dark) and environmental (light) factors currently known to be acting on chemical (green) and visual (blue) signals in the wood tiger moth. Anti-clockwise from top left: **(A)** Female neck defensive fluids are deterrent to avian predators; **(B)** Female melanisation is heritable and influenced by larval diet; **(C)** Female hindwing coloration is highly heritable, affected by larval diet, and influences avian predation risk; **(D)** Males follow female pheromone trails; **(E)** Male abdominal defensive fluids deter invertebrate predators; **(F)** Male hindwing colouration is heritable and plays a role in both predator deterrence and male mating success; **(G)** Male melanisation is heritable and influences both thermoregulation and predation risk; **(H)** Male neck defensive fluids are deterrent to avian predators. See text for details.

FIGURE 10 | Caucasian populations of the wood tiger moth are characterized by the lack of sexual dimorphism in hindwing colouration seen in Europe. In Georgia, both males **(A)** and females **(B)** exhibit hindwing coloration rich in long wavelengths resulting in continuous red-orange coloration. Photos: B. Rojas.

Nokelainen et al., 2012, 2014; Hegna and Mappes, 2014) and, lately, the same has been shown for its chemical defenses, even when experimentally decoupled from the visual signals (Brain, 2016; Rojas et al., 2017).

Male multimodal warning display has been shown to have important consequences for predator defense. A series of experiments using artificial moths showed that white males suffer higher predation rates in the field when compared to yellows (Nokelainen et al., 2014). Furthermore, when live moths were presented to birds, yellow males elicited longer attack latencies, suggesting yellow males possess stronger warning signals (Nokelainen et al., 2012). Yellow males seem to have more efficient chemical defenses against ants, and a more repulsive odor against avian predators when presented in isolation from the visual signal (Rojas et al., 2017), although the fluids of both morphs seem to be unpalatable even when presented in the absence of color cues. When the warning colors are presented to birds in association with the natural chemical defenses (the moth as a whole), however, white moths elicit more beak cleaning in great tits than yellow moths, and are also eaten less when attacked for the first time (Rönkä et al., 2018b). Thus, while yellow males seem to rely mostly on their warning color and repulsive odor to avoid being attacked, white males seem to rely on taste-rejection by predators, indicating that the multiple components of these moths' warning displays repel wild-caught predators at different stages of predation. Furthermore, white and yellow male color morphs trade-off between efficient warning and sexual signaling (see below). The white-colored males generally have a higher mating success (Nokelainen et al., 2012; Gordon et al., 2015) whereas the yellow-colored males are more avoided by avian predators (Nokelainen et al., 2012). Moreover, changes in the composition of the avian predator community can influence the direction of signal selection (Nokelainen et al., 2014), which, combined with spatial variation in differential mating success, may operate as a selection mosaic whereby dispersal facilitates the maintenance of genetic (Galarza et al., 2014), and hence phenotypic variation (Gordon et al., 2015).

Females are also well protected from predation. They, too, produce chemical defenses that deter birds effectively, but are costly to produce (Brain, 2016; Furlanetto, 2017). Red females are slightly more conspicuous (Lindstedt et al., 2011; Henze et al., 2018), and less frequently attacked by avian predators (Lindstedt et al., 2011) than orange ones, and experiments with wild-caught birds have demonstrated that the red coloration is learned faster than white and yellow (Rönkä et al., 2018a).

Why Are the European Forms Sexually Dimorphic? The Emerging Role of Sexual Selection

Sexual dimorphism, as well as sex-limited mimicry, are highly derived characters in many Lepidopteran systems (Kunte, 2008; Allen et al., 2010), but do occur multiple times in the Arctiinae. However, in Arctiina, the clade to which *A. plantaginis* belongs (Rönkä et al., 2016), sexual dimorphism is rare, suggesting that the ancestral state of the wood tiger moth is sexually monomorphic. Although the putative ancestral (Caucasian) forms of the species, as well as closely related species, exhibit a rather reddish coloration in both sexes (i.e., this population is not strictly sexually dichromatic; Rönkä et al., 2016; **Figure 10**), in a great portion of its range wood tiger moth morphs exhibit sexually dimorphic coloration (Hegna et al., 2015). Moreover, in several European populations white and yellow hind-winged males coexist locally, while females exhibit coloration that varies continuously from yellow through to orange and red.

Differences in reproductive allocation between males and females, and the subsequent differences in mate-searching behavior, can lead to differential exposure of the two sexes to predation. Female wood tiger moths, like many Lepidopteran females, allocate more resources to reproduction than to flight, eclosing with eggs ready to be fertilized. As is typical for moths, females use pheromones to attract males, who fly long distances in search of mates. Not surprisingly, males show their activity peak at the same time as the peak in female pheromone calling (Rojas et al., 2015a). Once they detect a female in the distance, they follow the pheromone source with a characteristic zig-zag flight. During the last stage of approach, it is also possible that males can detect the females visually, as these are particularly conspicuous against the vegetation on which they rest and call (Henze et al., 2018). Indeed, male eyes are more sensitive (Henze et al., 2018), which makes sense considering that they do most of the flying and maneuvering while searching for females. These ecological and behavioral differences between the sexes make it likely that the optimal values of signaling and defenses against predators are not the same for females and males.

Although natural selection can work on sexually dimorphic signals, and can both restrict or enhance the evolution of differences between sexes, sexual selection has been put forward as the main driving force of sexual dimorphism in Lepidoptera (Shine, 1989; Allen et al., 2010). A recent study examining the visual capabilities of both male and female wood tiger moths indicated that these moths are unable to distinguish among the different shades of orange-red that a female could have in its hindwings (Henze et al., 2018); this suggests that female coloration, as well as the coloration of Caucasian males, is unlikely to be influenced by sexual selection. Females, in contrast, are capable of distinguishing between the yellow and white coloration of Finnish male hindwings (Henze et al., 2018), pointing at a possible role of sexual selection, perhaps via female choice, on male hindwing polymorphism. Interestingly, some studies have revealed differences between the two male morphs in mating success, with white males getting a mating advantage, particularly when more abundant (Gordon et al., 2015) or when males are stressed/have costs imposed upon them (Nokelainen et al., 2012). Altogether, this hints at a role of coloration in mate choice or attraction, providing a possible explanation to wood tiger moths' sexual dimorphism in coloration.

Ongoing and future work including the investigation of the genetic mechanisms limiting the genetic correlation between sexes (e.g., sex-limited expression of autosomal genes can facilitate sexual dimorphism; Traut et al., 2007), quantifying costs and condition-dependence of sexually dimorphic traits and measuring natural and sexual selection in natural populations in the wood tiger moth system will continue to clarify the roles of both sexual and natural selection in the origins and maintenance of sexual dimorphism and male polymorphism.

Components of Chemical Defenses Could Have a Dual Function in Predator Deterrence and Mate Attraction

Recent developments concerning the chemical defenses of the wood tiger moth have revealed the presence of two methopyraxines (2-sec-butyl-3-methoxypyrazine and 2-isobutyl-3-methoxypyrazine) that have a key role in predator deterrence (Rojas et al., 2017; Burdfield-Steel et al., 2018). By contrast, despite luring *A. plantaginis* males to pheromone traps during every field season, our knowledge of the compounds present in the pheromone blend(s) of *A. plantaginis* is only incipient (Muraki et al., 2017), with a number of microcompounds amongst the most prominent components. Pyrazines have been found in the pheromone blends of some insects, and seem to be particularly common in tiger moths (Rothschild et al., 1984; Guilford et al., 1987; Moore et al., 1990). Thus, we cannot dismiss the possibility that these methopyraxines, or some other compounds found in the prothoracic defensive fluids of these moths, have a dual role in protection from predators and mate attraction. Furthermore, we know that males transfer a spermatophore to females during mating (Chargé et al., 2016), but we currently do not know whether some protective chemicals, or any other type of nuptial gift, are also transmitted during this transfer.

Studies on the interaction between warning coloration and chemical defenses in the context of sexual selection are the next logical steps in studies on wood tiger moths. They are an excellent model to test these different components because it is possible to test the effect of each in isolation to understand its function and importance. The amount of both methoxypyrazines can be measured from individual moths, allowing detailed estimates of chemical defense level. With our increasing knowledge of the pheromones of this species we can begin to look for links between pheromone composition and chemical defense, and in particular, if the resource allocation patterns seen in coloration and defense (Furlanetto, 2017), extend to pheromone production. In that respect, it is also important to discover whether males produce pheromones, as is the case in some butterflies (Darragh et al., 2017) and other day-flying moths (Sarto I Monteys et al., 2016), and whether those are relevant for female choice/acceptance. If males do have pheromones, it would be key to examine whether there are additive effects of pheromone blend and hindwing color, or whether one signal is more important than the sum of both for mate attraction/choice. The same question could be addressed in the context of population divergence, for instance to understand if potential variation in the pheromone blends and chemical defenses link to the differences in coloration between the European and Caucasian populations.

CURRENT KNOWLEDGE GAPS AND FUTURE DIRECTIONS

As our review shows, previous studies have been focusing on understanding how sexual or natural selection (seldom both; **Table 1**, **Figure 6C**) could shape the evolution of warning coloration due to its multiple function as a signal of mate quality and possession of chemical defenses. Our review also highlights the need to study a greater variety of "non-model" species, such as the wood tiger moth. In particular, species that possess key components such as chemical or visual, may help fill critical gaps in our existing knowledge. We describe some of these gaps below, and propose some future avenues of study.

1. Studies exploring how natural versus sexual selection affect primary defenses are not abundant, but are on the rise. In contrast, with a few exceptions (e.g., studies on the dual role of pyrrolizine alkaloids in bella moths *Utetheisa ornatrix*; Conner et al., 1981), less effort has been made to test the possible multiple functions of chemical compounds in chemical communication between conspecifics and in predator deterrence. One potential chemical group with multiple functions could be pyrazines, a group of compounds that are relatively common in insect defensive fluids (Rothschild et al., 1984; Guilford et al., 1987; Moore et al., 1990; Vencl et al., 2016; Rojas et al., 2017). It is possible that, for example, the intensity of the repulsive odor produced by pyrazines could also function as an honest signal of quality. In those terms, a male with higher concentration of pyrazines could be better protected against predators, which in turn would inform females that he has the right condition to afford the costs of production or sequestration and thus make him more attractive as a mate. To our knowledge, this has been studied in detail only in, *U. ornatrix* (González et al., 1999; Iyengar and Starks, 2008); see section "Chemical Compounds Can Play a Role in Mate Attraction and Predator Deterrence—But Could They Also Have a Dual Function?" above). Another way in which sexual and natural selection could act in synergy on chemical compounds is through the so-called nuptial gifts. A male could, for example, donate defensive chemicals to the female during mating to provide protection for the eggs or herself. In this type of situation the "odor" of the male could function both as a warning signal for predators and as a signal of mate quality for the females. Therefore, future research should consider the potential synergistic (or opposing) interactions between sexual selection and predation acting *simultaneously* on chemical and visual communication. We can start by investigating if the same compounds in the chemical defenses are also present in the pheromone blends, and how are they then potentially transferred to the spermatophores and eggs. We also need to test the relationship between the levels/types of defensive toxins a male possesses, combined with their attractiveness as a mate and their defensive coloration. Potentially good model organisms from which we already have information both on the influence of sexual and natural selection on different components of multimodal signals are listed in **Table 1**.

2. Defensive chemicals often evolve under multiple selection, protecting simultaneously from predators, pathogens, and parasitoids (Johnson Pieter et al., 2018). Many chemical compounds used in secondary defense or chemical communication can be sequestered directly from the diet or produced with the help of symbionts, which can alter the chemical profile of their hosts (Engl and Kaltenpoth, 2018). However, experimental evidence illustrating these interactions and their effect on host behaviors are still scarce. This is particularly true for symbionts involved in the production of insect pheromones (Engl and Kaltenpoth, 2018), thus providing a promising research avenue.

3. It is ideal to investigate the patterns of inheritance of the signals of interest. If the trait in question is not heritable, there are no grounds for natural selection to act on it (even if the trait is essential for survival). Likewise, we need to continue to study in depth how phenotypic variation exposed to selection by receivers is induced and maintained. To do that, we need to define the life-history costs of production and maintenance of different multimodal-signal components under various biotic and abiotic conditions (Hegna et al., 2013; Brain, 2016; Lindstedt et al., 2016). This will give us key information on how much of the signal variation is environmentally induced.

4. Before the role of any signal in either predator deterrence or mate attraction can be established, it is essential to identify and confirm the selective agent. Failure to properly do so can lead to misinterpretation or overestimation of the studied trait function. Chemical defenses, for example, may have very rich profiles with hundreds of compounds but, if relevant predators do not respond to them, then that defense is not under selection

by predators. It is of curse possible that the relevant predator is no longer present and we are thus witnessing the consequence of past selection. Furthermore, we should keep in mind that selective agents fluctuate across time and space (e.g., Endler and Rojas, 2009; Nokelainen et al., 2014). This means, for example, that identifying a predator at a particular location does not imply it is a selective agent elsewhere.

5. An integrative study of multimodal signal evolution should involve a better understanding of how signals are processed by the receiver's sensory systems. Recent advances in the field of visual ecology (e.g., animal vision models; Vorobyev and Osorio, 1998; Kelber et al., 2003; Endler and Mielke, 2005; Maia et al., 2013; Kemp et al., 2015; Troscianko and Stevens, 2015; Renoult et al., 2017; Maia and White, 2018), as well as well-known and widely used methods in chemical ecology (Harborne, 1997; Agelopoulos and Pickett, 1998) offer the tools to study if and how signals are discriminated against the background noise. However, knowing that a signal can be perceived is not enough. In addition to that, classic predation and mate choice experiments, particularly those in which the multiple components of the warning display can be tested in isolation and in different combinations, can provide information about the receiver's response. Whether or not receivers react to the signals sent by the emitter is what actually determines how these signals are shaped.

6. The role of behavior in aposematic displays has been largely understudied, although it has the potential to be, if not a signal, a relevant cue for predators in combination with other components. Evidence from studies on mimicry has highlighted how mimics fool predators by mimicking the motion type of their models. Such is the case of ant-mimicking spiders (genus *Myrmarachne*) of the family Salticidae, which are thought to mimic not only the morphology but also the characteristic movement of their ant models (Nelson and Card, 2016); or of certain species of hover flies (family Syrphidae), which mimic the behavior of wasps (Penney et al., 2014). While both the *Myrmarachne* spiders and the hover flies are Batesian (undefended) mimics, and thus not aposematic, they raise the question of whether aposematic species do also use behavior as a component of their warning displays. To date, we are aware of only one study in that direction: Neotropical aposematic butterflies can be told apart by bird predators from their non-aposematic counterparts on the basis of their flight behavior (Chai, 1986), and it cannot be discarded that it also plays a role in interactions between conspecifics, for example in courtship displays. Therefore, behavior in general, and motion in particular, combined with either warning colors or chemical defenses, may have a key function in predator deterrence and interactions between conspecifics in aposematic species.

7. Finally, the origin and spread of the first individuals bearing aposematic signals continues to be a matter of debate (Mappes et al., 2005; Speed and Ruxton, 2005). The first individuals with a conspicuous warning coloration would have been an easy target for predators, making it perplexing that they were able to multiply until they were numerous enough to prompt predator avoidance. One potential solution for this problem is that natural and sexual selection could both favor the evolution of aposematic displays, and one way to tackle it is using phylogenetic comparative methods. These methods have been used to study, for example, the correlated evolution of warning coloration and toxicity (Summers, 1987; Summers and Clough, 2001), but they would also be valuable to better understand how natural selection and sexual selection have jointly shaped the evolution of multimodal warning displays.

AUTHOR CONTRIBUTIONS

BR, JM, CL, SG, EB-S, KR, and ON conceived the scope of the review. BR, CD, and LH collected information from the literature. BR did the analyses and figures, and led the writing. All authors contributed to the discussion of ideas, writing and editing the manuscript, and approved the final version of it.

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REFERENCES

Adamova-Jezova, D., Hospodkova, E., Fuchsova, L., Stys, P., and Exnerova, A. (2016). Through experience to boldness? Deactivation of neophobia towards novel and aposematic prey in three European species of tits (Paridae). *Behav. Process.* 131, 24–31. doi: 10.1016/j.beproc.2016.07.014

Agelopoulos, N. G., and Pickett, J. A. (1998). Headspace analysis in chemical ecology: effects of different sampling methods on ratios of volatile compounds present in headspace samples. *J. Chem. Ecol.* 24, 1161–1172. doi: 10.1023/A:1022442818196

Aliabadi, A., Renwick, J. A., and Whitman, D. W. (2002). Sequestration of glucosinolates by harlequin bug *Murgantia histrionica*. *J. Chem. Ecol.* 28, 1749–1762. doi: 10.1023/A:1020505016637

Allen, C. E., Zwaan, B. J., and Brakefield, P. M. (2010). Evolution of sexual dimorphism in the Lepidoptera. *Annu. Rev. Entomol.* 56, 445–464. doi: 10.1146/annurev-ento-120709-144828

Aluthwattha, S. T., Harrison, R. D., Ranawana, K. B., Xu, C., Lai, R., and Chen, J. (2017). Does spatial variation in predation pressure modulate selection for aposematism? *Ecol. Evol.* 7, 7560–7572. doi: 10.1002/ece3.3221

Andersen, K. K., Bernstien, D. T., Caret, R. L., and Romanczyk, L. J. (1982). Chemical constituents of the defensive secretion of the striped skunk (*Mephitis mephitis*). *Tetrahedron* 38, 1965–1970. doi: 10.1016/0040-4020(82)80046-X

Ang, H. P., and Newman, L. J. (1998). Warning colouration in pseudocerotid flatworms (Platyhelminthes, Polycladida). A preliminary study. *Hydrobiologia* 383, 29–33. doi: 10.1023/A:1003449226586

Arias, M., Meichanetzoglou, A., Elias, M., Rosser, N., De-Silva, D. L., Nay, B., et al. (2016). Variation in cyanogenic compounds concentration within a *Heliconius* butterfly community: does mimicry explain everything? *BMC Evol. Biol.* 16:272. doi: 10.1186/s12862-016-0843-5

Awad, M., Laugier, G. J. M., Loiseau, A., and Nedvěd, O. (2015). Unbalanced polyandry in wild-caught ladybirds *Harmonia axyridis* (Coleoptera: Coccinellidae). *Appl. Entomol. Zool.* 50, 427–434. doi: 10.1007/s13355-015-0348-5

Barber, J. R., Chadwell, B. A., Garrett, N., Schmidt-French, B., and Conner, W. E. (2009). Naive bats discriminate arctiid moth warning sounds but generalize their aposematic meaning. *J. Exp. Biol.* 212, 2141–2148. doi: 10.1242/jeb.029991

Barber, J. R., and Conner, W. E. (2007). Acoustic mimicry in a predator-prey interaction. *Proc. Natl. Acad. Sci. U.S.A.* 104, 9331–9334. doi: 10.1073/pnas.0703627104

Benes, J., and Vesely, P. (2017). The ability of lizards to identify an artificial Batesian mimic. *Zoology* 123, 46–52. doi: 10.1016/j.zool.2017.05.010

Benson, W. W. (1972). Natural selection for Müllerian mimicry in *Heliconius erato* in Costa Rica. *Science* 176:936. doi: 10.1126/science.176.4037.936

Bezzerides, A. L., McGraw, K. J., Parker, R. S., and Husseini, J. (2007). Elytra color as a signal of chemical defense in the Asian ladybird beetle *Harmonia axyridis*. *Behav. Ecol. Sociobiol.* 61, 1401–1408. doi: 10.1007/s00265-007-0371-9

Bonacci, T., Aloise, G., Brandmayr, P., Brandmayr, T. Z., and Capula, M. (2008). Testing the predatory behaviour of *Podarcis sicula* (Reptilia : Lacertidae) towards aposematic and non-aposematic preys. *Amphib. Reptil.* 29, 449–453. doi: 10.1163/156853808785111986

Bonacci, T., Massolo, A., Brandmayr, P., and Brandmayr, T. Z. (2006). Predatory behaviour on ground beetles (Coleoptera : Carabidae) by *Ocypus olens* (Muller) (Coleoptera : Staphylinidae) under laboratory conditions. *Entomol. News* 117, 545–551. doi: 10.3157/0013-872X(2006)117[545:PBOGBC]2.0.CO;2

Boppré, M., Petty, R. L., Schneider, D., Meinwald, J. (1978). Behaviorally mediated contacts between scent organs: Another prerequisite for pheromone production in *Danaus chrysippus* males (lepidoptera). *J. Comp. Physiol.* 126, 97–103. doi: 10.1007/BF00666361

Borer, M., Van Noort, T., Rahier, M., and Naisbit, R. E. (2010). Positive frequency-dependent selection on warning color in alpine leaf beetles *Evolution* 64, 3629–3633. doi: 10.1111/j.1558-5646.2010.01137.x

Bowers, M. D. (1992). "The evolution of unpalatability and the cost of chemical defense in insects," in *Insect Chemical Ecology. An Evolutionary Approach,* eds B. D. Roitberg and M. B. Isman (London: Chapman & Hall), 216–244.

Bradbury, J. W., and Vehrencamp, S. L. (2011). *Principles of Animal Communication.* Sunderland, MA: Sinauer Associates Inc.

Brain, M. A. (2016). *Can Warning Signals Be Honest? Wing Colouration and the Strength of Chemical Defence in the Female Wood Tiger Moth.* University of Jyväskylä.

Brodie, E. D., and Janzen, F. J. (1995). Experimental studies of coral snake mimicry - generalized avoidance of ringed snake patterns by free-ranging avian predators. *Funct. Ecol.* 9, 186–190. doi: 10.2307/2390563

Brown, J. L., Maan, M. E., Cummings, M. E., and Summers, K. (2010). Evidence for selection on coloration in a Panamanian poison frog: a coalescent-based approach. *J. Biogeogr.* 37, 891–901. doi: 10.1111/j.1365-2699.2009.02260.x

Brown, Jr, K. S. (1984). Adult-obtained pyrrolizidine alkaloids defend ithomiine butterflies against a spider predator. *Nature* 309, 707.

Buasso, C. M., Leynaud, G. C., and Cruz, F. B. (2006). Predation on snakes of Argentina: effects of coloration and ring pattern on coral and false coral snakes. *Stud. Neotrop. Fauna Environ.* 41, 183–188. doi: 10.1080/01650520600630725

Burdfield-Steel, E., Pakkanen, H., Rojas, B., Galarza, J. A., and Mappes, J. (2018). *De novo* synthesis of chemical defenses in an aposematic moth. *J. Insect Sci.* 18:28. doi: 10.1093/jisesa/iey020

Burdfield-Steel, E. R., Dougherty, L. R., Smith, L. A., Collins, L. A., and Shuker, D. M. (2013). Variation in social and sexual behaviour in four species of aposematic seed bugs (Hemiptera: Lygaeidae): the role of toxic and non-toxic food. *Behav. Process.* 99, 52–61. doi: 10.1016/j.beproc.2013.06.006

Cardoso, M. Z. (1997). Testing chemical defence based on pyrrolizidine alkaloids. *Anim. Behav.* 54, 985–991. doi: 10.1006/anbe.1997.0505

Cardoso, M. Z., and Gilbert, L. E. (2013). Pollen feeding, resource allocation and the evolution of chemical defence in passion vine butterflies. *J. Evol. Biol.* 26, 1254–1260. doi: 10.1111/jeb.12119

Casas-Cardona, S., Marquez, R., and Vargas-Salinas, F. (2018). Different colour morphs of the poison frog *Andinobates bombetes* (Dendrobatidae) are similarly effective visual predator deterrents. *Ethology* 124, 245–255. doi: 10.1111/eth.12729

Castillo-Guevara, C., and Rico-Gray, V. (2002). Is cycasin in *Eumaeus minyas* (Lepiodoptera : Lycaenidae) a predator deterrent? *Interciencia* 27, 465–470.

Chai, P. (1986). Field observations and feeding experiments on the responses of Rufous-tailed jacamars (*Galbula ruficauda*) to free-flying butterflies in a tropical rainforest. *Biol. J. Linn. Soc.* 29, 161–189. doi: 10.1111/j.1095-8312.1986.tb01772.x

Chargé, R., Wedell, N., Lindstedt, C., Hämäläinen, L., Övermark, E., and Mappes, J. (2016). Variation in male fertility in a polymorphic moth, *Parasemia plantaginis*. *Anim. Behav.* 111, 33–40. doi: 10.1016/j.anbehav.2015.10.014

Chouteau, M., and Angers, B. (2011). The role of predators in maintaining the geographic organization of aposematic signals. *Am. Nat.* 178, 810–817. doi: 10.1086/662667

Chouteau, M., Arias, M., and Joron, M. (2016). Warning signals are under positive frequency-dependent selection in nature. *Proc. Natl. Acad. Sci. U.S.A.* 113, 2164–2169. doi: 10.1073/pnas.1519216113

Chouteau, M., Llaurens, V., Piron-Prunier, F., and Joron, M. (2017). Polymorphism at a mimicry supergene maintained by opposing frequency-dependent selection pressures. *Proc. Natl. Acad. Sci. U.S.A.* 114, 8325–8329. doi: 10.1073/pnas.1702482114

Codella, S. G., and Lederhouse, R. C. (1990). The effect of wing orientation on aposematic signaling in the pipevine swallowtail butterfly, *Battus philenor*. *Anim. Behav.* 40, 404–406. doi: 10.1016/S0003-3472(05)80938-8

Comeault, A. A., and Noonan, B. P. (2011). Spatial variation in the fitness of divergent aposematic phenotypes of the poison frog, *Dendrobates tinctorius*. *J. Evol. Biol.* 24, 1374–1379. doi: 10.1111/j.1420-9101.2011.02258.x

Conner, W. E., Eisner, T., Vander Meer, R. K., Guerrero, A., and Meinwald, J. (1981). Precopulatory sexual interaction in an arctiid moth (*Utetheisa ornatrix*): role of a pheromone derived from dietary alkaloids. *Behav. Ecol. Sociobiol.* 9, 227–235. doi: 10.1007/BF00302942

Cortesi, F., and Cheney, K. L. (2010). Conspicuousness is correlated with toxicity in marine opisthobranchs. *J. Evol. Biol.* 23, 1509–1518. doi: 10.1111/j.1420-9101.2010.02018.x

Cott, H. B. (1940). *Adaptive Colouration in Animals.* London: Methuen.

Crothers, L., Gering, E., and Cummings, M. (2011). Aposematic signal variation predicts male-male interactions in a polymorphic poison frog *Evolution* 65, 599–605. doi: 10.1111/j.1558-5646.2010.01154.x

Crothers, L. R., and Cummings, M. E. (2015). A multifunctional warning signal behaves as an agonistic status signal in a poison frog. *Behav. Ecol*. 26, 560–568. doi: 10.1093/beheco/aru231

Cummings, M. E., and Crothers, L. R. (2013). Interacting selection diversifies warning signals in a polytypic frog: an examination with the strawberry poison frog. *Evol. Ecol.* 27, 693–710. doi: 10.1007/s10682-013-9648-9

Darragh, K., Vanjari, S., Mann, F., Gonzalez-Rojas, M. F., Morrison, C. R., Salazar, C., et al. (2017). Male sex pheromone components in *Heliconius* butterflies released by the androconia affect female choice. *PeerJ* 5:e3953. doi: 10.7717/peerj.3953

Darst, C. R., Cummings, M. E., and Cannatella, D. C. (2006). A mechanism for diversity in warning signals: conspicuousness versus toxicity in poison frogs. *Proc. Natl. Acad. Sci. U.S.A.* 103:5852. doi: 10.1073/pnas.0600625103

Darwin, C. R. (1869). *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. London: John Murray.

Dell'aglio, D. D., Stevens, M., and Jiggins, C. D. (2016). Avoidance of an aposematically coloured butterfly by wild birds in a tropical forest. *Ecol. Entomol.* 41, 627–632. doi: 10.1111/een.12335

Dolenska, M., Nedved, O., Vesely, P., Tesarova, M., and Fuchs, R. (2009). What constitutes optical warning signals of ladybirds (Coleoptera: Coccinellidae) towards bird predators: colour, pattern or general look? *Biol. J. Linn. Soc.* 98, 234–242. doi: 10.1111/j.1095-8312.2009.01277.x

Dreher, C. E., and Pröhl, H. (2014). Multiple sexual signals: calls over colors for mate attraction in an aposematic, color-diverse poison frog. *Front. Ecol. Evol.* 2:22. doi: 10.3389/fevo.2014.00022

Dunning, D. C., Acharya, L., Merriman, C. B., and Dalferro, L. (1992). Interactions between bats and arctiid moths. *Can. J. Zool.* 70, 2218–2223. doi: 10.1139/z92-298

Dunning, D. C., and Kruger, M. (1995). Aposematic sounds in african moths. *Biotropica* 27, 227–231. doi: 10.2307/2388998

Dunning, D. C., and Kruger, M. (1996). Predation upon moths by free-foraging *Hipposideros caffer*. *J. Mammal.* 77, 708–715. doi: 10.2307/1382675

Edmunds, M. (1974). *Defence in Animals: A Survey of Antipredator Defences*. New York, NY: Longman.

Eisner, T., Schroeder, F. C., Snyder, N., Grant, J. B., Aneshansley, D. J., Utterback, D., et al. (2008). Defensive chemistry of lycid beetles and of mimetic cerambycid beetles that feed on them. *Chemoecology* 18, 109–119. doi: 10.1007/s00049-007-0398-4

Eisner, T., Smedley, S. R., Young, D. K., Eisner, M., Roach, B., and Meinwald, J. (1996a). Chemical basis of courtship in a beetle (*Neopyrochroa flabellata*): cantharidin as "nuptial gift". *Proc. Natl. Acad. Sci. U.S.A.* 93, 6499–6503. doi: 10.1073/pnas.93.13.6499

Eisner, T., Smedley, S. R., Young, D. K., Eisner, M., Roach, B., and Meinwald, J. (1996b). Chemical basis of courtship in a beetle (*Neopyrochroa flabellata*): cantharidin as precopulatory "enticing" agent. *Proc. Natl. Acad. Sci. U.S.A.* 93:6494. doi: 10.1073/pnas.93.13.6494

Endler, J. A. (1988a). Frequency-dependent predation, crypsis and aposematic coloration. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 319, 505–523. doi: 10.1098/rstb.1988.0062

Endler, J. A. (1988b). Sexual selection and predation risk in guppies. *Nature* 332, 593–594. doi: 10.1038/332593b0

Endler, J. A. (1992). Signals, signal condition and the direction of evolution. *Am. Nat.* 139, S125–S153. doi: 10.1086/285308

Endler, J. A., and Mappes, J. (2004). Predator mixes and the conspicuousness of aposematic signals. *Am. Nat.* 163, 532–547. doi: 10.1086/382662

Endler, J. A., and Mielke, P. W. (2005). Comparing entire colour patterns as birds see them. *Biol. J. Linn. Soc.* 86, 405–431. doi: 10.1111/j.1095-8312.2005.00540.x

Endler, J. A., and Rojas, B. (2009). The spatial pattern of natural selection when selection depends on experience. *Am. Nat.* 173, E62–E78. doi: 10.1086/596528

Engl, T., and Kaltenpoth, M. (2018). Influence of microbial symbionts on insect pheromones. *Nat. Prod. Rep.* 35, 386–397. doi: 10.1039/C7NP00068E

Estrada, C., and Jiggins, C. D. (2008). Interspecific sexual attraction because of convergence in warning colouration: is there a conflict between natural and sexual selection in mimetic species? *J. Evol. Biol.* 21, 749–760. doi: 10.1111/j.1420-9101.2008.01517.x

Exnerová, A., Jezová, D., Stys, P., Doktorovová, L., Rojas, B., and Mappes, J. (2015). Different reactions to aposematic prey in 2 geographically distant populations of great tits. *Behav. Ecol.* 26, 1361–1370. doi: 10.1093/beheco/arv086

Exnerová, A., Svadová, K., Stys, P., Barcalová, S., Landová, E., Prokopová, M., et al. (2006). Importance of colour in the reaction of passerine predators to aposematic prey: experiments with mutants of Pyrrhocoris apterus (Heteroptera). *Biol. J. Linn. Soc.* 88, 143–153. doi: 10.1111/j.1095-8312.2006.00611.x

Finkbeiner, S. D., Briscoe, A., and Reed, R. D. (2014). Warning signals are seductive: relative contributions of color and pattern to predator avoidance and mate attraction in *Heliconius* butterflies. *Evolution* 68, 3410–3420. doi: 10.1111/evo.12524

Fisher, R. A. (1930). *The Genetical Theory of Natural Selection*. Oxford: Oxford University Press.

Furlanetto, M. (2017). *Investigating the Costs of Visual Signals and Chemical Defences in Arctia plantaginis*. MSc Thesis, University of Padova.

Gade, M. R., Hill, M., and Saporito, R. A. (2016). Color assortative mating in a mainland population of the poison frog *Oophaga pumilio*. *Ethology* 122, 851–858. doi: 10.1111/eth.12533

Galarza, J. A., Nokelainen, O., Ashrafi, R., Hegna, R. H., and Mappes, J. (2014). Temporal relationship between genetic and warning signal variation in the aposematic wood tiger moth (*Parasemia plantaginis*). *Mol. Ecol.* 23, 4939–4957. doi: 10.1111/mec.12913

Geiselhardt, S., Otte, T., and Hilker, M. (2012). Looking for a similar partner: host plants shape mating preferences of herbivorous insects by altering their contact pheromones. *Ecol. Lett.* 15, 971–977. doi: 10.1111/j.1461-0248.2012.01816.x

Gimenez-Casalduero, F., Thacker, R. W., and Paul, V. J. (1999). Association of color and feeding deterrence by tropical reef fishes. *Chemoecology* 9, 33–39. doi: 10.1007/s000490050031

Gohli, J., and Hogstedt, G. (2009). Explaining the evolution of warning coloration: secreted secondary defence chemicals may facilitate the evolution of visual aposematic signals. *PLoS ONE* 4:e5779. doi: 10.1371/journal.pone.0005779

González, A., Rossini, C., Eisner, M., and Eisner, T. (1999). Sexually transmitted chemical defense in a moth *Utetheisa ornatrix*. *Proc. Natl. Acad. Sci. U.S.A.* 96:5570. doi: 10.1073/pnas.96.10.5570

Gordon, S. P., Kokko, H., Rojas, B., Nokelainen, O., and Mappes, J. (2015). Colour polymorphism torn apart by opposing positive frequency-dependent selection, yet maintained in space. *J. Anim. Ecol.* 84, 1555–1564. doi: 10.1111/1365-2656.12416

Groeters, F. R., and Strong, K. L. (1993). Observations on distastefulness of *Monistria concinna* (Walker) (Orthoptera, Pyrgomorphidae). *J. Austr. Entomol. Soc.* 32, 153–154. doi: 10.1111/j.1440-6055.1993.tb00564.x

Groot, A. T., Horovitz, J. L., Hamilton, J., Santangelo, R. G., Schal, C., and Gould, F. (2006). Experimental evidence for interspecific directional selection on moth pheromone communication. *Proc. Natl. Acad. Sci. U.S.A.* 103:5858. doi: 10.1073/pnas.0508609103

Groot, A. T., Inglis, O., Bowdridge, S., Santangelo, R. G., Blanco, C., Juan, D., et al. (2009). Geographic and temporal variation in moth chemical communication. *Evolution* 63, 1987–2003. doi: 10.1111/j.1558-5646.2009.00702.x

Guilford, T., Nicol, C., Rothschild, M., and Moore, B. P. (1987). The biological roles of pyrazines: evidence for a warning odour function. *Biol. J. Linn. Soc.* 31, 113–128. doi: 10.1111/j.1095-8312.1987.tb01984.x

Haber, M., Cerfeda, S., Carbone, M., Calado, G., Gaspar, H., Neves, R., et al. (2010). Coloration and defense in the nudibranch gastropod *Hypselodoris fontandraui*. *Biol. Bull.* 218, 181–188. doi: 10.1086/BBLv218n2p181

Hämäläinen, L., Valkonen, J., Mappes, J., and Rojas, B. (2015). Visual illusions in predator-prey interactions: birds find moving patterned prey harder to catch. *Anim. Cogn.* 18, 1059–1068. doi: 10.1007/s10071-015-0874-0

Harborne, J. B. (1997). Recent advances in chemical ecology. *Nat. Prod. Rep.* 14, 83–98. doi: 10.1039/np9971400083

Härlin, C., and Härlin, M. (2003). Towards a historization of aposematism. *Evol. Ecol.* 17, 197–212. doi: 10.1023/A:1023047930360

Hatle, J. D., and Faragher, S. G. (1998). Slow movement increases the survivorship of a chemically defended grasshopper in predatory encounters. *Oecologia* 115, 260–267. doi: 10.1007/s004420050515

Heath, R. R., Mclaughlin, J. R., Proshold, F., and Teal, P. E. A. (1991). Periodicity of female sex pheromone titer and release in Heliothis subflexa and *H. virescens* (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Am.* 84, 182–189. doi: 10.1093/aesa/84.2.182

Hebets, E. A., and Papaj, D. R. (2005). Complex signal function: developing a framework of testable hypotheses. *Behav. Ecol. Sociobiol.* 57, 197–214. doi: 10.1007/s00265-004-0865-7

Hegna, R. H., Galarza, J. A., and Mappes, J. (2015). Global phylogeography and geographical variation in warning coloration of the wood tiger moth (*Parasemia plantaginis*). *J. Biogeogr.* 42, 1469–1481. doi: 10.1111/jbi.12513

Hegna, R. H., and Mappes, J. (2014). Influences of geographic differentiation in the forewing warning signal of the wood tiger moth in Alaska. *Evol. Ecol.* 28, 1003–1017. doi: 10.1007/s10682-014-9734-7

Hegna, R. H., Saporito, R. A., and Donnelly, M. A. (2013). Not all colors are equal: predation and color polytypism in the aposematic poison frog *Oophaga pumilio*. *Evol. Ecol.* 27, 831–845. doi: 10.1007/s10682-012-9605-z

Hegna, R. H., Saporito, R. A., Gerow, K. G., and Donnelly, M. A. (2011). Contrasting colors of an aposematic poison frog do not affect predation. *Ann. Zool. Fenn.* 48, 29–38. doi: 10.5735/086.048.0103

Henze, M., Lind, O., Mappes, J., Rojas, B., and Kelber, A. (2018). An aposematic colour-polymorphic moth seen through the eyes of conspecifics and predators - sensitivity and colour discrimination in a tiger moth. *Funct. Ecol.* doi: 10.1111/1365-2435.13100. [Epub ahead of print].

Higham, J. P., and Hebets, E. A. (2013). An introduction to multimodal communication. *Behav. Ecol. Sociobiol.* 67, 1381–1388. doi: 10.1007/s00265-013-1590-x

Honma, A., Mappes, J., and Valkonen, J. K. (2015). Warning coloration can be disruptive: aposematic marginal wing patterning in the wood tiger moth. *Ecol. Evol.* 5, 4863–4874. doi: 10.1002/ece3.1736

Hristov, N., and Conner, W. E. (2005). Effectiveness of tiger moth (Lepidoptera, Arctiidae) chemical defenses against an insectivorous bat (*Eptesicus fuscus*). *Chemoecology* 15, 105–113. doi: 10.1007/s00049-005-0301-0

Hunter, J. S. (2009). Familiarity breeds contempt: effects of striped skunk color, shape, and abundance on wild carnivore behavior. *Behav. Ecol.* 20, 1315–1322. doi: 10.1093/beheco/arp144

Ingalls, V. (1993). Startle and habituation responses of blue jays (*Cyanocitta cristata*) in a laboratory simulation of antipredator defenses of catocala moths (Lepidoptera, Noctuidae). *Behaviour* 126, 77–96. doi: 10.1163/156853993X00353

Ingleby, F. C. (2015). Insect cuticular hydrocarbons as dynamic traits in sexual communication. *Insects* 6, 732–742. doi: 10.3390/insects6030732

Iyengar, V. K., and Starks, B. D. (2008). Sexual selection in harems: male competition plays a larger role than female choice in an amphipod. *Behav. Ecol.* 19, 642–649. doi: 10.1093/beheco/arn009

Jiggins, C. D. (2017). *The Ecology and Evolution of Heliconius Butterflies.* Oxford: Oxford University Press.

Jiggins, C. D., Naisbit, R. E., Coe, R. L., and Mallet, J. (2001). Reproductive isolation caused by colour pattern mimicry. *Nature* 411, 302–305. doi: 10.1038/35077075

Johansen, A. I., Exnerová, A., Svadová, K. H., Stys, P., Gamberale-Stille, G., and Tullberg, B. S. (2010). Adaptive change in protective coloration in adult striated shieldbugs *Graphosoma lineatum* (Heteroptera: Pentatomidae): test of detectability of two colour forms by avian predators. *Ecol. Entomol.* 35, 602–610. doi: 10.1111/j.1365-2311.2010.01219.x

Johnson Pieter, T. J., Calhoun Dana, M., Stokes Amber, N., Susbilla Calvin, B., McDevitt-Galles, T., Briggs Cheryl, J., et al. (2018). Of poisons and parasites—the defensive role of tetrodotoxin against infections in newts. *J. Anim. Ecol.* 87, 1192–1204. doi: 10.1111/1365-2656.12816

Joron, M., and Mallet, J. (1998). Diversity in mimicry: paradox or paradigm? . *Trends Ecol. Evol.* 13, 461–463. doi: 10.1016/S0169-5347(98)01483-9

Kang, C. K., Lee, S. I., and Jablonski, P. G. (2011). Effect of sex and bright coloration on survival and predator-induced wing damage in an aposematic lantern fly with startle display. *Ecol. Entomol.* 36, 709–716. doi: 10.1111/j.1365-2311.2011.01319.x

Kauppinen, J., and Mappes, J. (2003). Why are wasps so intimidating: field experiments on hunting dragonflies (Odonata : Aeshna grandis). *Anim. Behav.* 66, 505–511. doi: 10.1006/anbe.2003.2225

Kelber, A., Vorobyev, M., and Osorio, D. (2003). Animal colour vision - behavioural tests and physiological concepts. *Biol. Rev.* 78, 81–118. doi: 10.1017/S1464793102005985

Kemp, D. J., Herberstein, M. E., Fleishman, L. J., Endler, J. A., Bennett, A. T. D., Dyer, A. G., et al. (2015). An integrative framework for the appraisal of coloration in nature. *Am. Nat.* 185, 705–724. doi: 10.1086/681021

Kim, Y. H., Brown, G. B., and Mosher, H. S. (1975). Tetrodotoxin: occurrence in atelopid frogs of Costa Rica. *Science* 189, 151–152. doi: 10.1126/science.1138374

Kirchner, W. H., and Roschard, J. (1999). Hissing in bumblebees: an interspecific defence signal. *Insectes Soc.* 46, 239–243. doi: 10.1007/s000400050140

Klun, J. A., Plimmer, J. R., Bierl-Leonhardt, B. A., Sparks, A. N., Primiani, M., Chapman, O. L., et al. (1980). Sex pheromone chemistry of female corn earworm moth, Heliothis zea. *Chem. Ecol.* 6, 165–175. doi: 10.1007/BF00987535

Kotiaho, J., Alatalo Rauno, V., Mappes, J., Parri, S., and Rivero, A. (1998). Male mating success and risk of predation in a wolf spider: a balance between sexual and natural selection? *J. Anim. Ecol.* 67, 287–291. doi: 10.1046/j.1365-2656.1998.00192.x

Krall, B. S., Bartelt, R. J., Lewis, C. J., and Whitman, D. W. (1999). Chemical defense in the stink bug *Cosmopepla bimaculata*. *J. Chem. Ecol.* 25, 2477–2494. doi: 10.1023/A:1020822107806

Kuchta, S. R. (2005). Experimental support for aposematic coloration in the salamander *Ensatina eschscholtzii xanthoptica*: implications for mimicry of Pacific Newts. *Copeia* 2005, 265–271. doi: 10.1643/CH-04-173R

Kunte, K. (2008). Mimetic butterflies support Wallace's model of sexual dimorphism. *Proc. R. Soc. B Biol. Sci.* 275, 1617–1624. doi: 10.1098/rspb.2008.0171

Landová, E., Svadová, K. H., Fuchs, R., Stys, P., and Exnerová, A. (2017). The effect of social learning on avoidance of aposematic prey in juvenile great tits (*Parus major*). *Anim. Cogn.* 20, 855–866. doi: 10.1007/s10071-017-1106-6

Langham, G. M. (2004). Specialized avian predators repeatedly attack novel color morphs of *Heliconius* butterflies. *Evolution* 58, 2783–2787. doi: 10.1111/j.0014-3820.2004.tb01629.x

Lanier, G. N., Classon, A., Stewart, T., Piston, J. J., and Silverstein, R. M. (1980). IPS pini: the basis for interpopulational differences in pheromone biology. *J. Chem. Ecol.* 6, 677–687. doi: 10.1007/BF00987678

Lartviere, S., and Messier, F. (1996). Aposematic behaviour in the striped skunk, *Mephitis mephitis*. *Ethology* 102, 986–992. doi: 10.1111/j.1439-0310.1996.tb01176.x

Lawrence, J. P., Mahony, M., and Noonan, B. P. (2018). Differential responses of avian and mammalian predators to phenotypic variation in Australian Brood Frogs. *PLoS ONE* 13:e195446. doi: 10.1371/journal.pone.0195446

Lindstedt, C., Boncoraglio, G., Cotter, S., Gilbert, J., and Kilner, R. M. (2017). Aposematism in the burying beetle? Dual function of anal fluid in parental care and chemical defense. *Behav. Ecol.* 28, 1414–1422. doi: 10.1093/beheco/arx100

Lindstedt, C., Eager, H., Ihalainen, E., Kahilainen, A., Stevens, M., and Mappes, J. (2011). Direction and strength of selection by predators for the color of the aposematic wood tiger moth. *Behav. Ecol.* 22, 580–587. doi: 10.1093/beheco/arr017

Lindstedt, C., Schroderus, E., Lindström, L., Mappes, T., and Mappes, J. (2016). Evolutionary constraints of warning signals: a genetic trade-off between the efficacy of larval and adult warning coloration can maintain variation in signal expression. *Evolution* 70, 2562–2672. doi: 10.1111/evo.13066

Lindstedt, C., Talsma, J. H. R., Ihalainen, E., Lindstrom, L., and Mappes, J. (2010). Diet quality affects warning coloration indirectly: excretion costs in a generalist herbivore. *Evolution* 64, 68–78. doi: 10.1111/j.1558-5646.2009.00796.x

Lindström, L., Ahtiainen, J. J., Mappes, J., Kotiaho, J. S., Lyytinen, A., and Alatalo, R. V. (2005). Negatively condition dependent predation cost of a positively condition dependent sexual signalling. *J. Evol. Biol.* 19, 649–656. doi: 10.1111/j.1420-9101.2005.01043.x

Lindström, L., Alatalo, R. V., Lyytinen, A., and Mappes, J. (2001). Strong antiapostatic selection against novel rare aposematic prey. *Proc. Natl. Acad. Sci. U.S.A.* 98, 9181–9184. doi: 10.1073/pnas.161071598

Long, E. C., Hahn, T. P., and Shapiro, A. M. (2014). Variation in wing pattern and palatability in a female-limited polymorphic mimicry system. *Ecol. Evol.* 4, 4543–4552. doi: 10.1002/ece3.1308

Lyytinen, A., Brakefield, P. M., Lindström, L., and Mappes, J. (2004). Does predation maintain eyespot plasticity in *Bicyclus anynana*? *Proc. R. Soc. Lond. Ser. B Biol. Sci.* 271:279. doi: 10.1098/rspb.2003.2571

Maan, M. E., and Cummings, M. E. (2008). Female preferences for aposematic signal components in a polymorphic poison frog. *Evolution* 62, 2334–2345. doi: 10.1111/j.1558-5646.2008.00454.x

Maan, M. E., and Cummings, M. E. (2009). Sexual dimorphism and directional sexual selection on aposematic signals in a poison frog. *Proc. Natl. Acad. Sci. U.S.A.* 106, 19072–19077. doi: 10.1073/pnas.0903327106

Maan, M. E., and Cummings, M. E. (2012). Poison frog colors are honest signals of toxicity, particularly for bird predators. *Am. Nat.* 179, E1–E14. doi: 10.1086/663197

Maan, M. E., and Seehausen, O. (2011). Ecology, sexual selection and speciation. *Ecol. Lett.* 14, 591–602. doi: 10.1111/j.1461-0248.2011.01606.x

Maia, R., Eliason, C. M., Bitton, P.-P., Doucet, S. M., and Shawkey, M. D. (2013). pavo: an R package for the analysis, visualization and organization of spectral data. *Methods Ecol. Evol.* 4, 906–913. doi: 10.1111/2041-210X.12069

Maia, R., and White, T. E. (2018). Comparing colors using visual models. *Behav. Ecol.* 29, 649–659. doi: 10.1093/beheco/ary017

Majerus, M., Odonald, P., and Weir, J. (1982). Evidence for preferential mating in *Adalia bipunctata*. *Heredity* 49, 37–49. doi: 10.1038/hdy.1982.63

Mallet, J. (2007). Hybrid speciation. *Nature* 446:279. doi: 10.1038/nature05706

Mallet, J. (2010). Shift happens! Shifting balance and the evolution of diversity in warning colour and mimicry. *Ecol. Entomol.* 35, 90–104. doi: 10.1111/j.1365-2311.2009.01137.x

Mallet, J., and Barton, N. H. (1989). Strong natural selection in a warning-color hybrid zone. *Evolution* 43, 421–431. doi: 10.1111/j.1558-5646.1989.tb04237.x

Mallet, J., and Joron, M. (1999). Evolution of diversity in warning color and mimicry: polymorphisms, shifting balance, and speciation. *Annu. Rev. Ecol. Syst.* 30, 201–233. doi: 10.1146/annurev.ecolsys.30.1.201

Mallet, J., and Singer, M. C. (1987). Individual selection, kin selection, and the shifting balance in the evolution of warning colours: the evidence from butterflies. *Biol. J. Linn. Soc.* 32, 337–350. doi: 10.1111/j.1095-8312.1987.tb00435.x

Mappes, J., Marples, N., and Endler, J. A. (2005). The complex business of survival by aposematism. *Trends Ecol. Evol.* 20, 598–603. doi: 10.1016/j.tree.2005.07.011

Marek, P., Papaj, D., Yeager, J., Molina, S., and Moore, W. (2011). Bioluminescent aposematism in millipedes. *Curr. Biol.* 21, R680–R681. doi: 10.1016/j.cub.2011.08.012

Marples, N. M., Vanveelen, W., and Brakefield, P. M. (1994). The relative importance of color, taste and smell in the protection of an aposematic insect *Coccinella septempunctata*. *Anim. Behav.* 48, 967–974. doi: 10.1006/anbe.1994.1322

Martinez-Freiria, F., De Lanuza, G. P. I., Pimenta, A. A., Pinto, T., and Santos, X. (2017). Aposematism and crypsis are not enough to explain dorsal polymorphism in the Iberian adder. *Acta Oecol. Int. J. Ecol.* 85, 165–173. doi: 10.1016/j.actao.2017.11.003

Mavárez, J., Salazar, C. A., Bermingham, E., Salcedo, C., Jiggins, C. D., and Linares, M. (2006). Speciation by hybridization in *Heliconius* butterflies. *Nature* 441:868. doi: 10.1038/nature04738

Maynard Smith, J., and Harper, D. G. C. (2003). *Animal Signals.* Oxford: Oxford University Press.

Mayr, E. (1963). *Animal Species and Evolution*. Cambridge: Harvard University Press.

Medina, I., Wang, I. J., Salazar, C., and Amézquita, A. (2013). Hybridization promotes color polymorphism in the aposematic harlequin poison frog, *Oophaga histrionica*. *Ecol. Evol.* 3, 4388–4400. doi: 10.1002/ece3.794

Meredith, T. L., Cowart, J. D., Henkel, T. P., and Pawlik, J. R. (2007). The polychaete Cirriformia punctata is chemically defended against generalist coral reef predators. *J. Exp. Mar. Biol. Ecol.* 353, 198–202. doi: 10.1016/j.jembe.2007.08.023

Mérot, C., Frérot, B., Leppik, E., and Joron, M. (2015). Beyond magic traits: multimodal mating cues in *Heliconius* butterflies. *Evolution* 69, 2891–2904. doi: 10.1111/evo.12789

Merrill, R., Dasmahapatra, K., Davey, J., Dell'aglio, D., Hanly, J., Huber, B., et al. (2015). The diversification of Heliconius butterflies: what have we learned in 150 years? *J. Evol. Biol.* 28, 1417–1438. doi: 10.1111/jeb.12672

Merrill Richard, M., Chia, A., and Nadeau Nicola, J. (2014). Divergent warning patterns contribute to assortative mating between incipient Heliconius species. *Ecol. Evol.* 4, 911–917. doi: 10.1002/ece3.996

Merrill, R. M., Wallbank, R. W. R., Bull, V., Salazar, P. C. A., Mallet, J., Stevens, M., et al. (2012). Disruptive ecological selection on a mating cue. *Proc. R. Soc. B Biol. Sci.* 279:4907. doi: 10.1098/rspb.2012.1968

Mochida, K. (2011). Combination of local selection pressures drives diversity in aposematic signals. *Evol. Ecol.* 25, 1017–1028. doi: 10.1007/s10682-011-9471-0

Moore, B. P., Brown, W. V., and Rothschild, M. (1990). Methylalkylpyrazines in aposematic insects, their hostplants and mimics. *Chemoecology* 1, 43–51. doi: 10.1007/BF01325227

Moore, C. D., and Hassall, C. (2016). A bee or not a bee: an experimental test of acoustic mimicry by hoverflies. *Behav. Ecol.* 27, 1767–1774. doi: 10.1093/beheco/arw107

Moosman, P. R., Cratsley, C. K., Lehto, S. D., and Thomas, H. H. (2009). Do courtship flashes of fireflies (Coleoptera: Lampyridae) serve as aposematic signals to insectivorous bats? *Anim. Behav.* 78, 1019–1025. doi: 10.1016/j.anbehav.2009.07.028

Mosher, H. S., Fuhrman, F. A., Buchwald, H. D., and Fischer, H. G. (1964). Tarichatoxin-Tetrodotoxin: a potent neurotoxin. *Science* 144, 1100–1110. doi: 10.1126/science.144.3622.1100

Müller, F. (1879). Ituna and Thyridia: a remarkable case of mimicry in butterflies. *Trans. Entomol. Soc. Lond.* 1879, 20–29.

Muraki, Y., Yamakawa, R., Yamamoto, M., Naka, H., Honma, A., Mappes, J., et al. (2017). GC/FT-IR Analysis of novel 4,6,9-Triene and 2,4,6,9-Tetraene occurring in a female pheromone gland of *Arctia plantaginis* (Erebidae: Arctiinae). *Am. J. Anal. Chem.* 8, 645–656. doi: 10.4236/ajac.2017.810047

Nahrstedt, A., and Davis, R. H. (1983). Occurrence, variation and biosynthesis of the cyanogenic glucosides linamarin and lotaustralin in species of the Heliconiini (Insecta: Lepidoptera). *Compar. Biochem. Physiol. Part B* 75, 65–73. doi: 10.1016/0305-0491(83)90041-X

Nelson, X. J., and Card, A. (2016). Locomotory mimicry in ant-like spiders. *Behav. Ecol.* 27, 700–707. doi: 10.1093/beheco/arv218

Newcombe, D., Blount Jonathan, D., Mitchell, C., and Moore Allen, J. (2013). Chemical egg defence in the large milkweed bug, *Oncopeltus fasciatus*, derives from maternal but not paternal diet. *Entomol. Exp. Appl.* 149, 197–205. doi: 10.1111/eea.12128

Niskanen, M., and Mappes, J. (2005). Significance of the dorsal zigzag pattern of *Vipera latastei gaditana* against avian predators. *J. Anim. Ecol.* 74, 1091–1101. doi: 10.1111/j.1365-2656.2005.01008.x

Noboa, V., and Gillette, R. (2013). Selective prey avoidance learning in the predatory sea slug *Pleurobranchaea californica*. *J. Exp. Biol.* 216, 3231–3236. doi: 10.1242/jeb.079384

Noge, K., Prudic, K. L., and Becerra, J. X. (2012). Defensive roles of (E)-2-Alkenals and related compounds in Heteroptera. *J. Chem. Ecol.* 38, 1050–1056. doi: 10.1007/s10886-012-0166-y

Nokelainen, O. (2013). Many forms of the wood tiger moth (*Parasemia plantaginis*): selective heterogeneity favours polymorphic warning signals. *Jyväskylä Stud. Biol. Environment. Sci.* 259, 1–52.

Nokelainen, O., Hegna, R. H., Reudler, J. H., Lindstedt, C., and Mappes, J. (2012). Trade-off between warning signal efficacy and mating success in the wood tiger moth. *Proc. R. Soc. B Biol. Sci.* 279, 257–265. doi: 10.1098/rspb.2011.0880

Nokelainen, O., Lindstedt, C., and Mappes, J. (2013). Environment-mediated morph-linked immune and life-history responses in the aposematic wood tiger moth. *J. Anim. Ecol.* 82, 653–662. doi: 10.1111/1365-2656.12037

Nokelainen, O., Valkonen, J., Lindstedt, C., and Mappes, J. (2014). Changes in predator community structure shifts the efficacy of two warning signals in Arctiid moths. *J. Anim. Ecol.* 83, 598–605. doi: 10.1111/1365-2656.12169

Noonan, B. P., and Comeault, A. A. (2009). The role of predator selection on polymorphic aposematic poison frogs. *Biol. Lett.* 5, 51–54. doi: 10.1098/rsbl.2008.0586

O'Donald, P., and Majerus, M. E. N. (1984). Polymorphism of melanic ladybirds maintained by frequency-dependent sexual selection. *Biol. J. Linn. Soc.* 23, 101–111. doi: 10.1111/j.1095-8312.1984.tb00131.x

Ojala, K., Julkunen-Tiito, R., Lindstrom, L., and Mappes, J. (2005). Diet affects the immune defence and life-history traits of an Arctiid moth *Parasemia plantaginis*. *Evol. Ecol. Res.* 7, 1153–1170.

Oliver, J. C., Robertson, K. A., and Monteiro, A. (2009). Accommodating natural and sexual selection in butterfly wing pattern evolution. *Proc. R. Soc. B Biol. Sci.* 276, 2369–2375. doi: 10.1098/rspb.2009.0182

Osawa, N., and Nishida, T. (1992). Seasonal-variation in elytral color polymorphism in *Harmonia axyridis* (the ladybird beetle) - the role of nonrandom mating. *Heredity* 69, 297–307. doi: 10.1038/hdy.1992.129

Ozel, L. D., and Stynoski, J. L. (2011). Differences in escape behavior between a cryptic and an aposematic litter frog. *J. Herpetol.* 45, 395–398. doi: 10.1670/10-249.1

Parri, S., Alatalo, R. V., Kotiaho, J., and Mappes, J. (1997). Female choice for male drumming in the wolf spider *Hygrolycosa rubrofasciata*. *Anim. Behav.* 53, 305–312. doi: 10.1006/anbe.1996.0371

Partan, S., and Marler, P. (1999). Communication goes multimodal. *Science* 283, 1272–1273. doi: 10.1126/science.283.5406.1272

Partan, S. R., and Marler, P. (2005). Issues in the classification of multimodal communication signals. *Am. Nat.* 166, 231–245. doi: 10.1086/431246

Pasteels, J. M., and Rowellrahier, M. (1991). Proximate and ultimate causes for host plant influence on chemical defense of leaf beetles (Coleoptera, Chrysomelidae). *Entomol. Gen.* 15, 227–235. doi: 10.1127/entom.gen/15/1991/227

Peck, D. C. (2000). Reflex bleeding in froghoppers (Homoptera : Cercopidae): variation in behavior and taxonomic distribution. *Ann. Entomol. Soc. Am.* 93, 1186–1194. doi: 10.1603/0013-8746(2000)093[1186:RBIFHC]2.0.CO;2

Penney, H. D., Hassall, C., Skevington, J. H., Lamborn, B., and Sherratt, T. N. (2014). The relationship between morphological and behavioral mimicry in hover flies (Diptera: Syrphidae). *Am. Nat.* 183, 281–289. doi: 10.1086/674612

Pinheiro, C. E. G. (1996). Palatability and escaping ability in neotropical butterflies: tests with wild kingbirds (*Tyrannus melancholicus*, Tyrannidae). *Biol. J. Linn. Soc.* 59, 351–365. doi: 10.1111/j.1095-8312.1996.tb01471.x

Poulton, E. B. (1890). *The Colours of Animals: Their Meaning and Use.* London: Kegan Paul, Trench, Trubner.

Preissler, K., and Pröhl, H. (2017). The effects of background coloration and dark spots on the risk of predation in poison frog models. *Evol. Ecol.* 31, 683–694. doi: 10.1007/s10682-017-9903-6

Pröhl, H., and Hödl, W. (1999). Parental investment, potential reproductive rates, and mating system in the strawberry dart-poison frog, *Dendrobates pumilio*. *Behav. Ecol. Sociobiol.* 46, 215–220. doi: 10.1007/s002650050612

Pröhl, H., and Ostrowski, T. (2011). Behavioural elements reflect phenotypic colour divergence in a poison frog. *Evol. Ecol.* 25, 993–1015. doi: 10.1007/s10682-010-9455-5

Pröhl, H., Willink, B., and Hauswaldt, S. (2013). Geographic variation in sexual signals and behaviour in two species of poison frogs. *Evol. Ecol. Res.* 15, 667–687.

Prokopova, M., Vesely, P., Fuchs, R., and Zrzavy, J. (2010). The role of size and colour pattern in protection of developmental stages of the red firebug (*Pyrrhocoris apterus*) against avian predators. *Biol. J. Linn. Soc.* 100, 890–898. doi: 10.1111/j.1095-8312.2010.01463.x

Qvarnström, A., Rudh, A., Edstrom, T., Odeen, A., Lovlie, H., and Tullberg, B. S. (2014). Coarse dark patterning functionally constrains adaptive shifts from aposematism to crypsis in strawberry poison frogs. *Evolution* 68, 2793–2803. doi: 10.1111/evo.12487

Ratcliffe, J. M., and Nydam, M. L. (2008). Multimodal warning signals for a multiple predator world. *Nature* 455, 96–U59. doi: 10.1038/nature07087

Renoult, J. P., Kelber, A., and Schaefer, H. M. (2017). Colour spaces in ecology and evolutionary biology. *Biol. Rev.* 92, 292–315. doi: 10.1111/brv.12230

Reudler, J. H., Lindstedt, C., Pakkanen, H., Lehtinen, I., and Mappes, J. (2015). Costs and benefits of plant allelochemicals in herbivore diet in a multi enemy world. *Oecologia* 179, 1147–1158. doi: 10.1007/s00442-015-3425-0

Reynolds, R. G., and Fitzpatrick, B. M. (2007). Assortative mating in poison-dart frogs based on an ecologically important trait. *Evolution* 61, 2253–2259. doi: 10.1111/j.1558-5646.2007.00174.x

Rice, W. R., and Hostert, E. E. (2017). Laboratory experiments on speciation: what have we learned in 40 years? *Evolution* 47, 1637–1653. doi: 10.1111/j.1558-5646.1993.tb01257.x

Robertson, K. A., and Monteiro, A. (2005). Female *Bicyclus anynana* butterflies choose males on the basis of their dorsal UV-reflective eyespot pupils. *Proc. R. Soc. B Biol. Sci.* 272:1541. doi: 10.1098/rspb.2005.3142

Rojas, B. (2012). *The Apparent Paradox of Colour Variation in Aposematic Poison Frogs PhD (unpublished).* Ph.D. Dissertation, Deakin University.

Rojas, B., Burdfield-Steel, E., Pakkanen, H., Suisto, K., Maczka, M., Schulz, S., et al. (2017). How to fight multiple enemies: target-specific chemical defences in an aposematic moth. *Proc. R. Soc. B Biol. Sci.* 284:20171424. doi: 10.1098/rspb.2017.1424

Rojas, B., and Endler, J. A. (2013). Sexual dimorphism and intra-populational colour pattern variation in the aposematic frog *Dendrobates tinctorius*. *Evol. Ecol.* 27, 739–753. doi: 10.1007/s10682-013-9640-4

Rojas, B., Gordon, S. P., and Mappes, J. (2015a). Frequency-dependent flight activity in the colour polymorphic wood tiger moth. *Curr. Zool.* 61, 765–772. doi: 10.1093/czoolo/61.4.765

Rojas, B., Rautiala, P., and Mappes, J. (2014). Differential detectability of polymorphic warning signals under varying light environments. *Behav. Process.* 109, 164–172. doi: 10.1016/j.beproc.2014.08.014

Rojas, B., Valkonen, J. K., and Nokelainen, O. (2015b). Aposematism. *Curr. Biol.* 25, R350–R351. doi: 10.1016/j.cub.2015.02.015

Rönkä, K., De Pasqual, C., Mappes, J., Gordon, S., and Rojas, B. (2018a). Colour alone matters: no predator generalization among morphs of an aposematic moth. *Anim. Behav.* 135, 153–163. doi: 10.1016/j.anbehav.2017.11.015

Rönkä, K., Mappes, J., Kaila, L., and Wahlberg, N. (2016). Putting *Parasemia* in its phylogenetic place: a molecular analysis of the subtribe Arctiina (Lepidoptera). *Syst. Entomol.* 41, 844–853. doi: 10.1111/syen.12194

Rönkä, K., Mappes, J., Kivi,ö, R., Salokannas, J., Michalis, C., and Rojas, B. (2018b). Can multiple-model mimicry explain warning signal polymorphism in the wood tiger moth, *Arctia plantaginis* (Lepidoptera: Erebidae)? *Biol. J. Linn. Soc.* 124, 237–260. doi: 10.1093/biolinnean/bly042

Roper, T. J. (1994). Conspicuousness of prey retards reversal of learned avoidance. *Oikos* 69, 115–118. doi: 10.2307/3545290

Roper, T. J., and Redston, S. (1987). Conspicuousness of distasteful prey affects the strength and durability of one-trial avoidance learning. *Anim. Behav.* 35, 739–747. doi: 10.1016/S0003-3472(87)80110-0

Roque-Albelo, L., Schroeder, F. C., Conner, W. E., Bezzerides, A., Hoebeke, E. R., Meinwald, J., et al. (2002). Chemical defense and aposematism: the case of *Utetheisa galapagensis*. *Chemoecology* 12, 153–157. doi: 10.1007/s00012-002-8341-6

Rothschild, M., Aplin, R. T., Cockrum, P. A., Edgar, J. A., Fairweather, P., and Lees, R. (1979). Pyrrolizidine alkaloids in arctiid moths (Lep.) with a discussion on host plant relationships and the role of these secondary plant substances in the Arctiidae. *Biol. J. Linn. Soc.* 12, 305–326. doi: 10.1111/j.1095-8312.1979.tb00062.x

Rothschild, M., Moore, B. P., and Brown, W. V. (1984). Pyrazines as warning odour components in the Monarch butterfly, *Danaus plexippus*, and in moths of the genera *Zygaena* and *Amata* (Lepidoptera). *Biol. J. Linn. Soc.* 23, 375–380. doi: 10.1111/j.1095-8312.1984.tb00153.x

Rowe, C. (1999). Receiver psychology and the evolution of multicomponent signals. *Anim. Behav.* 58, 921–931. doi: 10.1006/anbe.1999.1242

Rowe, C., and Guilford, T. (1999). The evolution of multimodal warning displays. *Evol. Ecol.* 13, 655–671. doi: 10.1023/A:1011021630244

Rowe, C., and Halpin, C. (2013). Why are warning displays multimodal? *Behav. Ecol. Sociobiol.* 67, 1425–1439. doi: 10.1007/s00265-013-1515-8

Rudh, A., Breed, M. F., and Qvarnstrom, A. (2013). Does aggression and explorative behaviour decrease with lost warning coloration? *Biol. J. Linn. Soc.* 108, 116–126. doi: 10.1111/j.1095-8312.2012.02006.x

Rudh, A., Rogell, B., Hastad, O., and Qvarnstrom, A. (2011). Rapid population divergence linked with co-variation between coloration and sexual display in strawberry poison frogs. *Evolution* 65, 1271–1282. doi: 10.1111/j.1558-5646.2010.01210.x

Ruxton, G. D., Sherratt, T. N., and Speed, M. P. (2004). *Avoiding Attack: The Evolutionary Ecology of Crypsis, Warning Signals and Mimicry*. Oxford: Oxford University Press.

Ryan, M. J., Tuttle, M. D., and Rand, A. S. (1982). Bat predation and sexual advertisement in a Neotropical anuran. *Am. Nat.* 119, 136–139. doi: 10.1086/283899

Salazar, C., Baxter, S. W., Pardo-Diaz, C., Wu, G., Surridge, A., Linares, M., et al. (2010). Genetic evidence for hybrid trait speciation in *Heliconius* butterflies. *PLoS Genet.* 6:e1000930. doi: 10.1371/journal.pgen.1000930

Santos, J. C., Baquero, M., Barrio-Amorós, C., Coloma, L. A., Erdtmann, L. K., Lima, A. P., et al. (2014). Aposematism increases acoustic diversification and speciation in poison frogs. *Proc. R. Soc. B Biol. Sci.* 281:20141761. doi: 10.1098/rspb.2014.1761

Santos, J. C., Coloma, L. A., and Cannatella, D. C. (2003). Multiple, recurring origins of aposematism and diet specialization in poison frogs. *Proc. Natl. Acad. Sci. U.S.A.* 100, 12792–12797. doi: 10.1073/pnas.2133521100

Santos, J. C., Tarvin, R. D., and O'Connell, L. A. (2016). "A review of chemical defense in poison frogs (dendrobatidae): ecology, pharmacokinetics, and autoresistance," in *Chemical Signals in Vertebrates 13,* eds B. Schulte, T. Goodwin, and M. Ferkin (Springer), 305–337.

Saporito, R. A., Donnelly, M. A., Spande, T. F., and Garraffo, H. M. (2012). A review of chemical ecology in poison frogs. *Chemoecology* 22, 159–168. doi: 10.1007/s00049-011-0088-0

Saporito, R. A., Zuercher, R., Roberts, M., Gerow, K. G., and Donnelly, M. A. (2007). Experimental evidence for aposematism in the dendrobatid poison frog *Oophaga pumilio*. *Copeia* 2007, 1006–1011. doi: 10.1643/0045-8511(2007)7[1006:EEFAIT]2.0.CO;2

Sarto I Monteys, V., Quero, C., Santa-Cruz, M. C., Rosell, G., and Guerrero, A. (2016). Sexual communication in day-flying Lepidoptera with special reference to castniids or 'butterfly-moths'. *Bull. Entomol. Res.* 106, 421–431. doi: 10.1017/S0007485316000158

Schultz, T. D. (2001). Tiger beetle defenses revisited: Alternative defense strategies and colorations of two neotropical tiger beetles, Odontocheila nicaraguensis Bates and Pseudoxycheila tasalis Bates (Carabidae : Cicindelinae). *Coleopt. Bull.* 55, 153–163. doi: 10.1649/0010-065X(2001)055[0153:TBDRAD]2.0.CO;2

Schultz, T. D., and Puchalski, J. (2001). Chemical defenses in the tiger beetle *Pseudoxycheila tarsalis* Bates (Carabidae : Cicindelinae). *Coleopt. Bull.* 55, 164–166. doi: 10.1649/0010-065X(2001)055[0164:CDITTB]2.0.CO;2

Servedio, M. (2009). The role of linkage disequilibrium in the evolution of premating isolation. *Heredity* 102, 51. doi: 10.1038/hdy.2008.98

Seybold, S. J., Quilici, D. R., Tillman, J. A., Vanderwel, D., Wood, D. L., and Blomquist, G. J. (1995). *De novo* biosynthesis of the aggregation pheromone components ipsenol and ipsdienol by the pine bark beetles *Ips paraconfusus* Lanier and *Ips pini* (Say) (Coleoptera: Scolytidae). *Proc. Natl. Acad. Sci. U.S.A.* 92:8393. doi: 10.1073/pnas.92.18.8393

Sharma Manmohan, D., Hunt, J., and Hosken David, J. (2011). Antagonistic responses to natural and sexual selection and the sex-specific evolution of cuticular hydrocarbons in *Drosophila simulans*. *Evolution* 66, 665–677. doi: 10.1111/j.1558-5646.2011.01468.x

Shine, R. (1989). Ecological causes for the evolution of sexual dimorphism - a review of the evidence. *Q. Rev. Biol.* 64, 419–461. doi: 10.1086/416458

Siddall, E. C., and Marples, N. M. (2011). Hear no evil: the effect of auditory warning signals on avian innate avoidance, learned avoidance and memory. *Curr. Zool.* 57, 197–207. doi: 10.1093/czoolo/57.2.197

Siddiqi, A., Cronin, T. W., Loew, E. R., Vorobyev, M., and Summers, K. (2004). Interspecific and intraspecific views of color signals in the strawberry poison frog *Dendrobates pumilio*. *J. Exp. Biol.* 207, 2471–2485. doi: 10.1242/jeb.01047

Speed, M. P., and Ruxton, G. D. (2005). Aposematism: what should our starting point be? *Proc. R. Soc. B Biol. Sci.* 272, 431–438. doi: 10.1098/rspb.2004.2968

Srygley, R. B. (1999). Locomotor mimicry in Heliconius butterflies: contrast analyses of flight morphology and kinematics. *Philos. Trans. R. Soc. B Biol. Sci.* 354, 203–214. doi: 10.1098/rstb.1999.0372

Stelzer, R. J., Raine, N. E., Schmitt, K. D., and Chittka, L. (2010). Effects of aposematic coloration on predation risk in bumblebees? A comparison between differently coloured populations, with consideration of the ultraviolet. *J. Zool.* 282, 75–83. doi: 10.1111/j.1469-7998.2010.00709.x

Stevens, M., and Ruxton, G. D. (2012). Linking the evolution and form of warning coloration in nature. *Philos. Trans. R. Soc. B Biol. Sci.* 279, 417–426. doi: 10.1098/rspb.2011.1932

Stuart, Y. E., Dappen, N., and Losin, N. (2012). Inferring predator behavior from attack rates on prey-replicas that differ in conspicuousness. *PLoS ONE* 7:e48497. doi: 10.1371/journal.pone.0048497

Su, S. Y., Lim, M., and Kunte, K. (2015). Prey from the eyes of predators: color discriminability of aposematic and mimetic butterflies from an avian visual perspective. *Evolution* 69, 2985–2994. doi: 10.1111/evo.12800

Summers, K. (1987). Reproductive strategies in the green poison-dart frog, *Dendrobates auratus*. *Am. Zool.* 27:A28.

Summers, K., and Clough, M. E. (2001). The evolution of coloration and toxicity in the poison frog family (Dendrobatidae). *Proc. Natl. Acad. Sci. U.S.A.* 98, 6227–6232. doi: 10.1073/pnas.101134898

Summers, K., Symula, R., Clough, M., and Cronin, T. (1999). Visual mate choice in poison frogs. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* 266, 2141–2145. doi: 10.1098/rspb.1999.0900

Svadová, K. H., Exnerová, A., Kopecková, M., and Stys, P. (2013). How do predators learn to recognize a mimetic complex: experiments with naive great tits and aposematic Heteroptera. *Ethology* 119, 814–830. doi: 10.1111/eth.12121

Sword, G. A., Simpson, S. J., El Hadi, O. T. M., and Wilps, H. (2000). Density-dependent aposematism in the desert locust. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* 267, 63–68. doi: 10.1098/rspb.2000.0967

Tabadkani, S. M., and Nozari, J. (2014). Relaxed predation hinders development of anti-predator behaviors in an aposematic beetle. *Entomol. Exp. Appl.* 153, 199–206. doi: 10.1111/eea.12241

Teal, P., Tumlinson, J., McLaughlin, J., Heath, R., and Rush, R. (1984). (Z)-11-Hexadecen-1-OL: a behavioral modifying chemical present in the pheromone gland of female *Heliothis zea* (Lepidoptera: Noctuidae). *Can. Entomol.* 116, 777–779. doi: 10.4039/Ent116777-5

Traut, W., Sahara, K., and Marec, F. (2007). Sex chromosomes and sex determination in Lepidoptera. *Sexual Dev.* 1, 332–346. doi: 10.1159/000111765

Troscianko, J., and Stevens, M. (2015). Image calibration and analysis toolbox – a free software suite for objectively measuring reflectance, colour and pattern. *Methods Ecol. Evol.* 6, 1320–1331. doi: 10.1111/2041-210X.12439

Tullrot, A. (1994). The evolution of unpalatability and warning coloration in soft-bodied marine invertebrates. *Evolution* 48, 925–928. doi: 10.1111/j.1558-5646.1994.tb01374.x

Tullrot, A., and Sundberg, P. (1991). The conspicuous nudibranch *Polycera quadrilineata* - aposematic coloration and individual selection. *Anim. Behav.* 41, 175–176. doi: 10.1016/S0003-3472(05)80513-5

Tuttle, M. D., and Ryan, M. J. (1981). Bat predation and the evolution of frog vocalizations in the neotropics. *Science* 214, 677–678. doi: 10.1126/science.214.4521.677

Ueno, H., Sato, Y., and Tsuchida, K. (1998). Colour-associated mating success in a polymorphic Ladybird Beetle, *Harmonia axyridis*. *Funct. Ecol.* 12, 757–761. doi: 10.1046/j.1365-2435.1998.00245.x

Valkonen, J. K., Nokelainen, O., Niskanen, M., Kilpimaa, J., Bjorklund, M., and Mappes, J. (2012). Variation in predator species abundance can cause variable selection pressure on warning signaling prey. *Ecol. Evol.* 2, 1971–1976. doi: 10.1002/ece3.315

Vencl, F. V., Ottens, K., Dixon, M. M., Candler, S., Bernal, X. E., Estrada, C., et al. (2016). Pyrazine emission by a tropical firefly: an example of chemical aposematism? *Biotropica* 48, 645–655. doi: 10.1111/btp.12336

Vesely, P., Vesela, S., Fuchs, R., and Zrzavy, J. (2006). Are gregarious red-black shieldbugs, *Graphosoma lineatum* (Hemiptera : Pentatomidae), really aposematic? An experimental approach. *Evol. Ecol. Res.* 8, 881–890.

Vidal-Cordero, J. M., Moreno-Rueda, G., Lopez-Orta, A., Marfil-Daza, C., Ros-Santaella, J. L., and Ortiz-Sanchez, F. J. (2012). Brighter-colored paper wasps (*Polistes dominula*) have larger poison glands. *Front. Zool.* 9:20. doi: 10.1186/1742-9994-9-20

Vorobyev, M., and Osorio, D. (1998). Receptor noise as a determinant of colour thresholds. *Proc. R. Soc. B Biol. Sci.* 265, 351–358. doi: 10.1098/rspb.1998.0302

Wang, I. J., and Shaffer, H. B. (2008). Rapid color evolution in an aposematic species: a phylogenetic analysis of color variation in the strikingly polymorphic strawberry poison-dart frog *Evolution* 62, 2742–2759. doi: 10.1111/j.1558-5646.2008.00507.x

Wang, I. J., and Summers, K. (2010). Genetic structure is correlated with phenotypic divergence rather than geographic isolation in the highly polymorphic strawberry poison-dart frog. *Mol. Ecol.* 19, 447–458. doi: 10.1111/j.1365-294X.2009.04465.x

Weller, S. J., Jacobson, N. L., and Conner, W. E. (1999). The evolution of chemical defences and mating systems in tiger moths (Lepidoptera: Arctiidae). *Biol. J. Linn. Soc.* 68, 557–578. doi: 10.1111/j.1095-8312.1999.tb01188.x

Willink, B., Brenes-Mora, E., Bolanos, F., and Prohl, H. (2013). Not everything is black and white: color and behavioral variation reveal a continuum between cryptic and aposematic strategies in a polymorphic poison frog. *Evolution* 67, 2783–2794. doi: 10.1111/evo.12153

Willink, B., Garcia-Rodriguez, A., Bolanos, F., and Prohl, H. (2014). The interplay between multiple predators and prey colour divergence. *Biol. J. Linn. Soc.* 113, 580–589. doi: 10.1111/bij.12355

Winters, A. E., Green, N. F., Wilson, N. G., How, M. J., Garson, M. J., Marshall, N. J., et al. (2017). Stabilizing selection on individual pattern elements of aposematic signals. *Proc. R. Soc. B Biol. Sci.* 284:20170926. doi: 10.1098/rspb.2017.0926

Winters, A. E., White, A. M., Dewi, A. S., Mudianta, I. W., Wilson, N. G., Forster, L. C., et al. (2018). Distribution of defensive metabolites in nudibranch molluscs. *J. Chem. Ecol.* 44, 384–396. doi: 10.1007/s10886-018-0941-5

Wuster, W., Allum, C. S. E., Bjargardottir, I. B., Bailey, K. L., Dawson, K. J., Guenioui, J., et al. (2004). Do aposematism and Batesian mimicry require bright colours? A test, using European viper markings. *Proc. R. Soc. B Biol. Sci.* 271, 2495–2499. doi: 10.1098/rspb.2004.2894

Yang, Y., Richards-Zawacki, C. L., Devar, A., and Dugas, M. B. (2016). Poison frog color morphs express assortative mate preferences in allopatry but not sympatry. *Evolution* 70, 2778–2788. doi: 10.1111/evo.13079

Yoshimura, Y., and Kasuya, E. (2013). Odorous and non-fatal skin secretion of adult wrinkled frog (*Rana rugosa*) is effective in avoiding predation by snakes. *PLoS ONE* 8:e81280. doi: 10.1371/journal.pone.0081280

Zagrobelny, M., Bak, S., Rasmussen, A. V., Jørgensen, B., Naumann, C. M., and Lindberg Møller, B. (2004). Cyanogenic glucosides and plant–insect interactions. *Phytochemistry* 65, 293–306. doi: 10.1016/j.phytochem.2003.10.016

Zuk, M., and Kolluru, G. R. (1998). Exploitation of sexual signals by predators and parasitoids. *Q. Rev. Biol.* 73, 415–438. doi: 10.1086/420412

Zylinski, S., and Osorio, D. (2013). Visual contrast and color in rapid learning of novel patterns by chicks. *J. Exp. Biol.* 216, 4184. doi: 10.1242/jeb.085001

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Noise Affects Multimodal Communication During Courtship in a Marine Fish

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Selection pressures on signals can be substantially modified by a changing environment, but we know little about how modified selection pressures act on multimodal signals. The currently increasing levels of anthropogenic noise in the ocean may affect the use of acoustic signaling relative to other modalities. In the Painted Goby (*Pomatoschistus pictus*), visual and acoustic signals are associated during courtship behavior, but females usually rely more heavily on acoustic signals than on visual signals in mate choice. In an aquarium experiment, we compared male courtship behavior and female spawning decisions between silent treatments and treatments with additional noise. We found that the relationships between male characteristics, male visual and acoustic courtship, and spawning success were affected by noise. A path analysis revealed that females pay more attention to visual courtship in noisy circumstances compared to control. We conclude that environmental stressors can cause shifts in the use of different signaling modalities for spawning decisions and discuss how selection pressures on multimodal signals may change with increasing noise-levels.

Keywords: acoustic communication, aquatic noise pollution, courtship behavior, multimodal shift, sexual selection, mate choice, *pomatoschistus pictus*, spawning

INTRODUCTION

The degree to which a signal is effective in carrying information to a receiver can be substantially modified by a changing environment. Loss of signal efficacy in changing environments may lead to signals being misunderstood or not received at all. To overcome a loss of information transfer, signaling individuals may adjust their signaling behavior by adapting the signal to the environment. For example, Tokay Geckos (*Gekko gecko*) have been found to increase the duration of their typically brief call notes in a noisy environment, in order to make them more easily heard (Brumm and Zollinger, 2017). Alternatively, signaling individuals may switch to different modalities that are not, or are less affected by environmental changes, such as found in tree frogs that produce more visual signals when background noise is high (Grafe et al., 2012). In addition, in those cases where the receiver benefits from the information contained in the signal (e.g., mate choice), the receiver may also adapt to a loss of signal efficacy by switching its attention to alternative signals or cues, such as stickleback females that pay more attention to chemical than visual signals in a turbid environment (Heuschele et al., 2009).

In fish, acoustic communication is known to play an important role in mating behavior and reproduction (Myrberg and Lugli, 2006). Acoustic signals may be essential for mate attraction and mate selection as seen in the Lusitanian Toadfish (*Halobatrachus didactylus*), a fish species where mate attraction and reproductive success depends on the male's acoustic courtship performance (Amorim et al., 2016). Sound production is also common in many gadoids and is thought to synchronize gamete release in Haddock, *Melanogrammus aeglefinus* (Hawkins and Amorim, 2000; Casaretto et al., 2015), and in Cod (*Gadus morhua*; Rowe and Hutchings, 2006).

Anthropogenic noise is a growing environmental concern, in particular in relation to aquatic life (Slabbekoorn et al., 2010; Popper et al., 2014; Radford et al., 2014). Aquatic animals may be particularly dependent on acoustic communication which overlaps in frequency with anthropogenic noise (Slabbekoorn et al., 2010; van der Sluijs et al., 2011; Radford et al., 2014). Noise can affect the range over which fish can communicate effectively (Vasconcelos et al., 2007; Alves et al., 2016; Stanley et al., 2017), and has been shown to affect acoustic signaling and reproductive behavior in fish (Picciulin et al., 2010, 2012; Bruintjes and Radford, 2013; Holt and Johnston, 2015; Nedelec et al., 2017; de Jong et al., 2018). Because noise can affect both signaling behavior and the propagation of signals, it is likely to affect the way information is transferred to the female during courtship. Therefore, noise could affect sexual selection and, as a result, change the frequency of certain acoustic traits in a population. For example, if male acoustic signals are rendered less effective as a source of information about male quality, females may stop paying attention to acoustic signals, leading to reduced sexual selection for this trait, which could, ultimately, result in trait-loss (Järvenpää and Lindstrom, 2004; Candolin et al., 2007; Tuomainen and Candolin, 2011).

Many animals rely on more than one modality to signal their quality: they may, for example, use sound and visual cues (Rowe, 1999; Hebets and Papaj, 2005). A main hypothesis for the function of multimodal signals is that one modality may be a back-up for a loss of signal efficacy in another modality (Bradbury and Vehrencamp, 1998; Hebets and Papaj, 2005). Such signal redundancy could mitigate effects of noise on acoustic signaling (Brumm and Slabbekoorn, 2005; van der Sluijs et al., 2011; Partan, 2017). However, such an effect would depend on whether and how the receiver uses the information it obtained from these different signals. In Painted Gobies (*Pomatoschistus pictus*), males lure females to their nests with both visual and acoustic signals, but females have been found to rely more heavily on acoustic than on visual signals for mate choice (Amorim et al., 2013). Therefore, it is an ideal model species to test how noise could affect multimodal communication. In this study, we tested whether this differential use of modalities changed when mating couples were exposed to noise during courtship and spawning. Under the hypothesis that females would pay less attention to signaling in the acoustic modality when this modality is disturbed by noise, we predicted that acoustic signaling would become less important for mating success than visual courtship under noisy conditions.

MATERIALS AND METHODS

General Design

The experiment was carried out in January and February 2015 at the University of Lisbon. We exposed male gobies to a control (noise-insulated aquaria) or an added noise treatment for 3 days. Females were introduced in a separate compartment within the same aquarium on the evening of day 3 to allow them to habituate to the acoustic environment. On day 4 we removed the partition to release the females into the male compartment and allowed free interaction and spawning. Each male was presented with two free-swimming females and we recorded male courtship behavior and female spawning behavior (added-noise: $N = 20$, control: $N = 16$).

Ethics

All experiments were performed in compliance with laws of Portugal. We operated under a permit for catching Painted Gobies from the National Defense Ministry (Autoridade Marítima Nacional-Capitania do Porto de Cascais), permit nr. 550/2013.

Study Species

The Painted Goby, (*P. pictus*), is a coastal marine species. This small benthic species inhabits shallow gravel and sand substrate areas in the Eastern Atlantic Ocean and in some areas of the Mediterranean Sea (Miller, 1986). The Painted Goby has a polygamous mating system, in which males build nests under bivalve shells, by shoveling sand in a pile over the shell (Bouchereau et al., 2003). Males attract females to spawn and take care of the eggs until they hatch. Males can take care of eggs of several females at the same time and in batches over the season. Males display both visually and acoustically during courtship (Amorim and Neves, 2007). Courtship vocalizations consist of drums and thumps (Amorim and Neves, 2007; Amorim et al., 2013).

Catching and Husbandry

Painted Gobies were caught in January and February 2015 with hand nets in intertidal pools at Parede (38° 41′N, 9° 21′W), Portugal. In the laboratory, males and females were kept separately in recirculated artificial sea water (32–35‰) under a 12 h: 12 h dark/ light regime at 16°C. Fish were fed twice a day *ad libitum* with a mix of chopped mussel, clams and shrimp. For detailed methods see de Jong et al. (2018).

Experimental Set-Up

Experimental aquaria contained a 3 cm layer of sand and a nest made from a PVC tube with a chimney to accommodate the hydrophone (**Figure 1**; see Amorim et al., 2013 and de Jong et al., 2018 for further details of the nest and recording setup). The nest was covered inside with a bendable plastic sheet for later

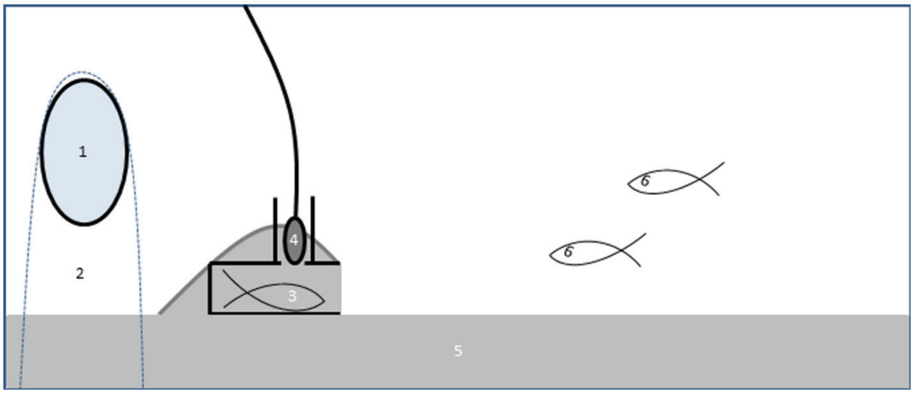

FIGURE 1 | Schematic overview of the experimental set-up in an experiment to test effects of noise on multimodal communication in the Painted Goby. A noise-egg (1) was placed in a cloth with sand as weight (2) behind the male's nest (3). A hydrophone in the nest chimney (4) recorded male sounds. Aquaria contained a layer of sand (5) on the bottom, which males used to build a nest over the provided PVC-tube. The male and the two females (6) could interact freely during the trials.

removal and photography of the eggs in the nest. The "noise egg," consisting of an electromotor in a waterproof container, was used to generate a constant low frequency multi-tone with a fundamental frequency around 100 Hz and several strong harmonics (de Jong et al., 2017, 2018) The background noise level in the control treatment was 100 ± 1 dB re 1 μPa ($N = 16$) compared to 125 ± 6 dB re 1 μPa ($N = 20$) in the added noise treatments [see (de Jong et al., 2018) for details]. Particle acceleration, measured with an accelerometer (see Klein et al., 2013; de Jong et al., 2018), was elevated on average by 20 dB at 200 Hz (i.e., around the main frequency of courtship drums; Amorim et al., 2013) compared to ambient recordings in the male nest. The harmonic structure of the experimental noise allowed us to unambiguously quantify the number of calls in the added noise treatment as well as in the control treatment. The noise-egg was placed just behind the nest in a cloth bag and weighed down with a stone. In the control treatments, the egg was switched off. Males of both species were allowed to acclimate to the treatment (*added noise* or *control*) for 3 days (day 1–3). On day 2 a stimulus female was introduced behind a partition to stimulate nest building, she was removed on day 3. Painted Gobies built nests by shoveling sand in a pile over the plastic tube.

Test females were measured to the nearest 0.5 mm, weighed to the nearest 0.1 g and introduced to a separate compartment in the experimental aquarium on the evening of day 3. They were allowed to acclimate overnight to the treatment for 12 h. During the acclimation period they could interact with the male behind a transparent partition at 30 cm from the male nest. On day 4, an hour before the start of the trial, we added an opaque divider to obtain a resting period without courtship. We started a trial by lifting the divider to release the females. We recorded acoustic and visual courtship for 30 min and noted whether and when either of the females entered the nest. After the trial, both male and females were left in the aquarium for what was left of day 4 and the morning of day 5, during which we checked the nest for eggs every 3 daylight-hours with a handheld torch. On day 5, we ended the trial and weighed males and females to the nearest 0.1 g, measured them to the nearest 0.5 mm and took a picture of the eggs on the plastic sheet. Male size was on average 41.6 ± 2.99 mm ($N = 36$) and weight was on average 0.63 ± 0.13 g. We used Fulton's K as a measure of condition, which was calculated by dividing the wet weight in g by the cubic of the length in cm times 100 (Ricker, 1975). Condition was on average 0.86 ± 0.06 ($N = 36$). There were no significant differences between the treatments in male total length (t-test: $t = 0.20$, $df = 28.7$, $P = 0.8$) or weight (t-test: $t = 0.87$, $df = 31.4$, $P = 0.4$), but there was a trend for males to have a lower condition at the end of the noise treatment compared to the control (t-test: $t = 2.04$, $df = 29.6$, $P = 0.05$).

Analyses

Sound analyses were done using PRAAT version 6.0.19. (Boersma and Weenink, 2017). We counted the number of drums and thumps made during the first 30 min of a trial. Visual courtship was scored from a silent video by an observer that was blind to the treatment (KdJ). Because the minimum time for a female to enter the nest to spawn was as short as 1 min and all visual courtship was completed before spawning, we only report counts for the first minute of visual courtship (as in de Jong et al., 2018). We counted the frequency of hops (the male approaches a female with short hop-like swimming motions), jumps (the male swims over or in front of the female and lands facing the other way, as a component of an eight-display), quivers (shaking the body), leads (the male swims towards the nest waving its tail in a characteristic manner), and fast approaches (including nudges: the male swims quickly toward the female in a straight line, sometimes nudging her in the side) (cf. Amorim and Neves, 2007). We also noted the number of longer swims that were not directed at a female. Female-female interactions (fast approaches) were very scarce and, therefore, left out of the analyses.

Statistical analyses were performed in R 3.4.3 (R Core Team, 2017). We used a simplified form of path analysis, because it is a useful way to visualize changes in relationships within the data. We proposed a single predicted path including all measured male characteristics (**Figure 2**) and we tested each relationship (arrow) within the path separately for the control and the added noise treatment. We tested each relationship

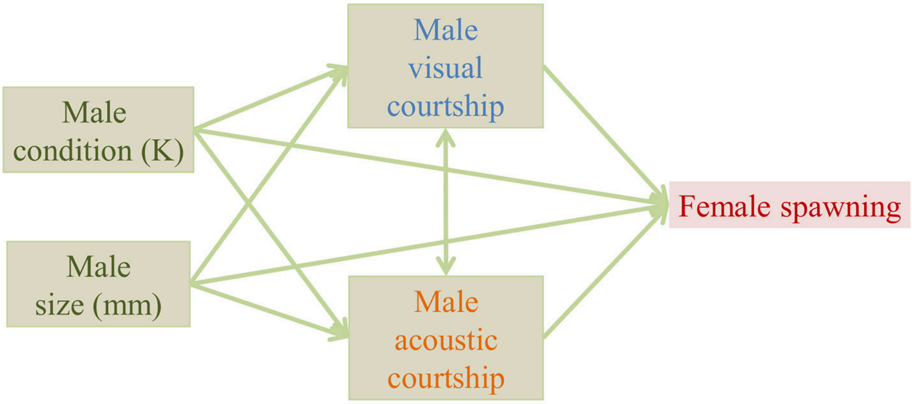

FIGURE 2 | Proposed path of measured variables potentially affecting the likelihood of female spawning in a Painted Goby aquarium experiment.

separately, because our sample size did not allow the inclusion of all effects in the same model. We tested the correlation between visual and acoustic courtship with a Spearman's rank correlation test. For all other steps we used generalized linear models with appropriate residual error structures [R packages: lme4 (R Core Team, 2017), MASS (Venables and Ripley, 2002)]. We used a quasibinomial error structure (glm, family = quasibinomial) for the effect of courtship frequency (acoustic and visual) and male characteristics (size and condition) on the likelihood of female spawning. We used Gaussian models (lm) for effects of male characteristics on the log-transformed visual courtship frequency, and we used a quasi-Poisson error structure (glm, family = quasipoisson) for the effects of male characteristics on acoustic courtship frequency. Model fit was verified by visual inspection of the residual plots provided in the plot function in lme4 and we report model results with and without outliers based on Cook's distance. In the model to test the effect of male size on acoustic courtship frequency, we found one data point with a Cook's distance > 1. After removal of this outlier, the estimation of the effect changed from 0.19 (CI: -0.04 to 0.42) to 0.35 (CI: 0.07 to 0.64), and thus from borderline non-significant to borderline significant. However, because we had no *a priori* reasons to exclude this outlier, and it did not change the results qualitatively, we chose to keep the outlier in the model. We provide estimates and confidence intervals for treatment effects in the figures, for full models see Supplementary Tables 1–8). If the confidence intervals of the estimated effect do not overlap with 0, the effect is significantly different from zero ($P < 0.05$).

RESULTS

Painted Gobies produced on average 1.5 ± 2 ($N = 36$) sounds per minute in a 30 min trial and displayed on average 19 ± 9.2 (mean ± SD, $N = 36$) visual behaviors in the first minute. Overall differences between the treatments in male visual and male acoustic behavior have been reported in a previous article (de Jong et al., 2018); the frequencies of both behaviors decreased in the added noise treatment.

As in previous studies (Amorim et al., 2013) the likelihood of successful spawning was predominantly correlated with the frequency of male acoustic courtship in the control treatment (**Figure 3A**). In the treatment with added noise, the frequency of male acoustic courtship still had a significant effect on spawning success, but male visual courtship frequency also had a significant positive effect (**Figure 3B**). Furthermore, male acoustic courtship frequency was significantly correlated with male visual courtship frequency and male size had a significant effect on male visual courtship frequency (**Figure 3B**).

The differences between the treatments were most pronounced for average or lower courtship frequencies (**Figure 4**). Males with high visual or acoustic courtship frequencies were predicted by the model to be successful in both treatments. However, males with lower visual courtship frequencies were less likely to spawn in the added noise treatment than in the control. Males with a lower acoustic courtship frequency, on the other hand, were more likely to spawn in the added noise treatment compared to the control (**Figure 4**).

DISCUSSION

We found that noise affects the relationship between male courtship behavior and female spawning decisions in the Painted Goby. While acoustic courtship frequency was the only significant predictor of spawning success in the control treatment, male visual courtship frequency was also a significant predictor of spawning success in the added noise treatment. The model predictions (**Figure 4**) showed that low acoustic activity is associated with a higher spawning likelihood in the additional noise treatment when compared to the control, while for visual courtship the opposite pattern is seen. Overall this suggests that visual courtship becomes more important in mating communication when noise disturbs acoustic communication in the Painted Goby.

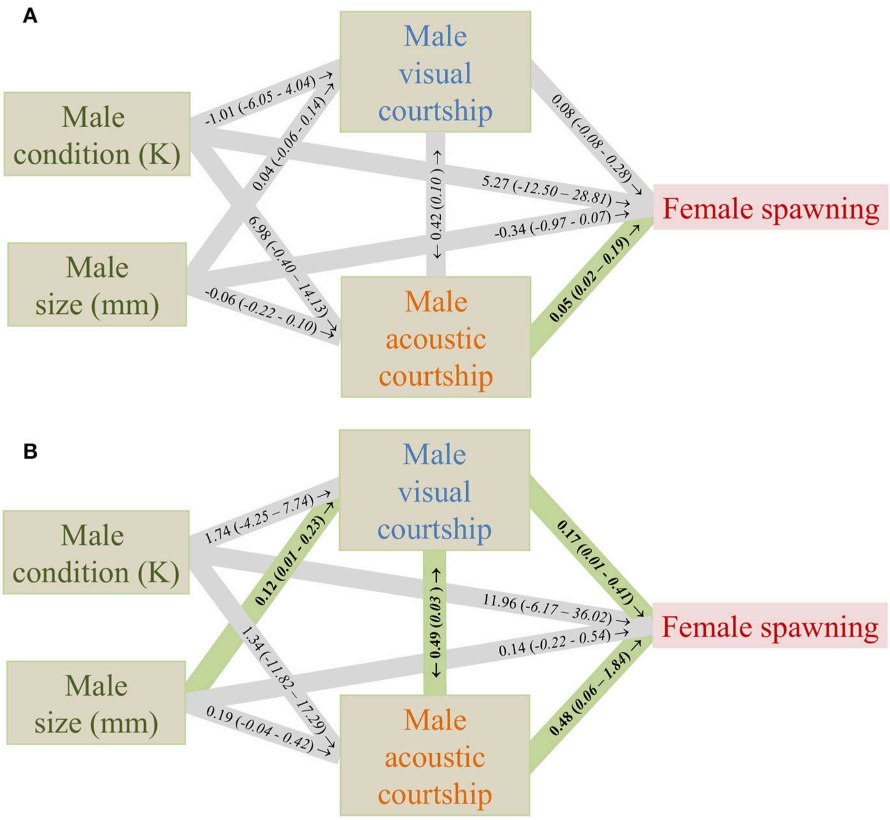

FIGURE 3 | Effects of measured variables on the likelihood of female spawning in an aquarium experiment in the Painted Goby in a control **(A)** and an added noise **(B)** treatment ($N = 16, 20$). Numbers in the lines are effect sizes from generalized linear models with their confidence intervals between brackets. See Supplementary Files for full model reports. The correlation between acoustic and visual courtship was tested with a Spearman's rank test, and therefore the rho is given with the *P*-value in brackets. Numbers in bold are significant effects ($P < 0.05$).

These results could be explained by the sensory compensation hypothesis, which states that multimodal signals may provide a back-up for information loss if the signal components in different modalities are redundant (Hartman and Abrahams, 2000; Hebets and Papaj, 2005; Bro-Jørgensen, 2010; Partan, 2017). If the efficacy of a certain signal component is reduced by noise in one of the modalities, both the signaler and the receiver may shift their communication efforts to another modality, which has been termed a multimodal shift (Partan et al., 2010). Examples of multimodal shifts have been found in all taxa, from invertebrates to mammals (reviewed in Partan, 2017). From the back-up hypothesis, an increase in visual signaling would have been expected under added noise conditions to compensate for the deterioration of acoustic communication, but in the current study male Painted Gobies did not increase visual signaling (de Jong et al., 2018). Nevertheless, females apparently paid more attention to visual signaling in the added noise treatment. A similar mismatch between male and female adjustments was found in the three-spined stickleback (*Gasterosteus aculeatus*), where males displayed more visual courtship in turbid conditions, while females paid more attention to chemical cues (Candolin et al., 2007; Heuschele et al., 2009). In the three-spined stickleback, this mismatch co-occurred with a weakened sexual selection on visual traits, which could ultimately lead to trait loss in the population (Candolin et al., 2007; Tuomainen and Candolin, 2011).

In addition to the relationship between visual courtship and spawning success, the relationships between visual courtship and acoustic courtship, and the relationship between male size and visual courtship were significant in the additional noise treatment (**Figure 3B**). The relationship between male size and acoustic courtship was borderline non-significant. One could suspect that this increase in the number of significant relationships in the additional noise treatment compared to the control was caused by an increase in the precision of the model estimates due to the larger sample size in the noise relative to the control treatment (20 vs. 16). Instead, the confidence intervals increased in all cases where we found a significant effect in the noise treatment that was non-significant in the control. This suggests that the increase in the number of significant relationships was caused by an increase in the actual effect sizes, and not by an increase in the precision of the model estimates. Call characteristics, including acoustic courtship frequency of male Painted Gobies have been previously correlated with male quality (Amorim et al., 2013) and may allow females to choose the best mates and also to distinguish

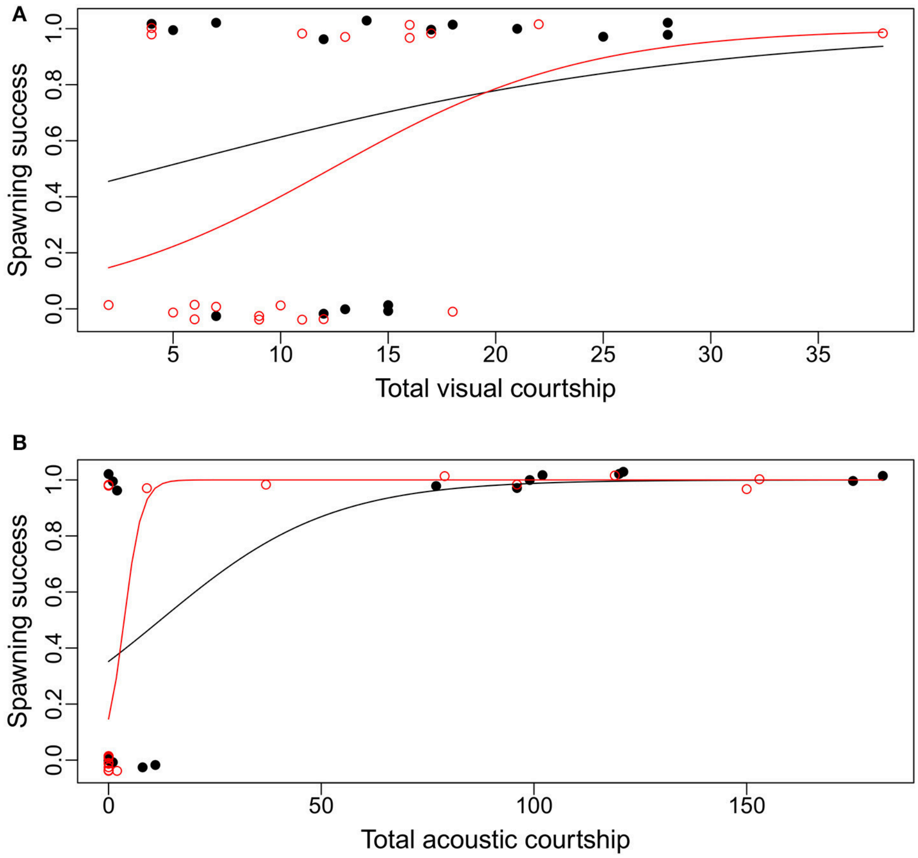

FIGURE 4 | The relationships between visual **(A)** and acoustic **(B)** courtship frequency and the probability of spawning success for Painted Goby males in an aquarium experiment to test the effect of additional noise on spawning decisions. Dots are individual males that either did (1) or did not (0) receive eggs from females in the control treatment (black dots) vs. additional noise (red circles). Lines are the model estimates from generalized linear models for the relationship between courtship frequency and spawning success in control (black) vs. additional noise (red). See Supplementary Files for full model reports.

between closely-related cryptic species (Pedroso et al., 2012). If such the information in such signals does not reach the female, because the propagation is hampered or because of masking, females may switch to more simple cues of male quality, such as size, which may be assessed directly (if visibility allows) and is most easily advertised in visual courtship. Such a mechanism could potentially explain the stronger relationship between male size, visual courtship frequency and spawning success in the additional noise treatment compared to the control.

Overall, we found a change in the importance of different modalities during mating interactions of the Painted Goby in response to increased noise levels. Although the reliance on acoustic courtship was maintained, visual courtship gained importance in the interactions between males and females under noisy conditions. In addition, male size became significantly associated with visual courtship frequency. Environmental changes have the potential to drastically alter sexual selection on traits (Miller and Svensson, 2014). We show that noise can change relationships between traits, signaling components in different modalities, and spawning success, which is the precursor for such changes in sexual selection. Future studies should focus on whether such changes result in a loss or change of acoustic traits in natural populations.

DATA AVAILABILITY STATEMENT

All data used for this paper is included in the Supplementary Table 9.

AUTHOR CONTRIBUTIONS

KdJ and KH designed the experiment, with comments from CA and PF. KdJ executed the experiment, analyzed the data and wrote the manuscript with comments and revisions by KH, CA, and PF.

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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.2018.00113/full#supplementary-material

REFERENCES

Alves, D., Amorim, M. C., and Fonseca, P. J. (2016). Assessing acoustic communication active space in the Lusitanian toadfish. *J. Exp. Biology* 219, 1122–1129. doi: 10.1242/jeb.134981

Amorim, M. C., Conti, C., Sousa-Santos, C., Novais, B., Gouveia, M. D., Vicente, J. R., et al. (2016). Reproductive success in the Lusitanian toadfish: influence of calling activity, male quality and experimental design. *Physiol. Behav.* 155, 17–24. doi: 10.1016/j.physbeh.2015.11.033

Amorim, M. C. P., Pedroso, S. S., Bolgan, M., Jordão, J. M., Caiano, M., and Fonseca, P. J. (2013). Painted gobies sing their quality out loud: acoustic rather than visual signals advertise male quality and contribute to mating success. *Funct. Ecol.* 27, 289–298. doi: 10.1111/1365-2435.12032

Amorim, M., and Neves, A. (2007). Acoustic signaling during courtship in the painted goby, *Pomatoschistus pictus*. *J. Marine Biol. Assoc.* 87, 1017–1023. doi: 10.1017/S0025315407056822

Boersma, P., and Weenink, D. (2017). *PRAAT*: *D*oing *P*honetics by *C*omputer.Available online at: www.praat.org

Bouchereau, J.-L., Houder, V., Marques, A., and Rebelo, J. (2003). A new distribution record and the reproductive strategy of *Pomatoschistu pictus* adriaticus (Pisces: Gobiidae) in the Mediterranean Sea. *J. Marine Biol. Assoc.* 83, 1157–1161. doi: 10.1017/S0025315403008427h

Bradbury, J. W., and Vehrencamp, S. L., (1998). *Principles of Animal Communication*. Sunderland, MA: Sinauer Associates, Inc.

Bro-Jørgensen, J. (2010). Dynamics of multiple signalling systems: animal communication in a world in flux. *Trends Ecol. Evol.* 25, 292–300. doi: 10.1016/j.tree.2009.11.003

Bruintjes, R., and Radford, A. N. (2013). Context-dependent impacts of anthropogenic noise on individual and social behaviour in a cooperatively breeding fish. *Anim. Behav.* 85, 1343–1349. doi: 10.1016/j.anbehav.2013.03.025

Brumm, H., and Slabbekoorn, H. (2005). Acoustic communication in noise. *Adv. Study Behav.* 35, 151–209. doi: 10.1016/S0065-3454(05)35004-2

Brumm, H., and Zollinger, S. A. (2017). Vocal plasticity in a reptile. *Proc. Biol. Sci. B* 284:20170451. doi: 10.1098/rspb.2017.0451

Candolin, U., Salesto, T., and Evers, M. (2007). Changed environmental conditions weaken sexual selection in sticklebacks. *J. Evol. Biol.* 20, 233–239. doi: 10.1111/j.1420-9101.2006.01207.x

Casaretto, L., Picciulin, M., and Hawkins, A. D. (2015). Mating behaviour by the haddock (*Melanogrammus aeglefinus*). *Environ. Biol. Fishes* 98, 913–923. doi: 10.1007/s10641-014-0327-7

de Jong, K., Amorim, M. C. P., Fonseca, P. J., Fox, C. J., and Heubel, K. U. (2018). Noise can affect acoustic communication and subsequent spawning success in fish. *Environ. Pollut.* 237, 814–823. doi: 10.1016/j.envpol.2017.11.003

de Jong, K., Schulte, G., and Heubel, K. U. (2017). The noise egg: a cheap and simple device to produce low-frequency underwater noise for laboratory and field experiments. *Methods Ecol. Evol.* 8, 268–274. doi: 10.1111/2041-210X.12653

Grafe, T. U., Preininger, D., Sztatecsny, M., Kasah, R., Dehling, J. M., Proksch, S., et al. (2012). Multimodal communication in a noisy environment: a case study of the Bornean rock frog *Staurois parvus*. *PLoS ONE* 7:e37965. doi: 10.1371/journal.pone.0037965

Hartman, E. J., and Abrahams, M. V. (2000). Sensory compensation and the detection of predators: the interaction between chemical and visual information. *Proc. Biol. Sci. B* 267, 571–575. doi: 10.1098/rspb.2000.1039

Hawkins, A., and Amorim, M. (2000). Spawning sounds of the male haddock, *Melanogrammus aeglefinus*. *Environ. Biol. Fishes* 59, 29–41. doi: 10.1023/A:1007615517287

Hebets, E. A., and Papaj, D. R. (2005). Complex signal function: developing a framework of testable hypotheses. *Behav. Ecol. Sociobiol.* 57, 197–214. doi: 10.1007/s00265-004-0865-7

Heuschele, J., Mannerla, M., Gienapp, P., and Candolin, U. (2009). Environment-dependent use of mate choice cues in sticklebacks. *Behav. Ecol.* 20, 1223–1227. doi: 10.1093/beheco/arp123

Holt, D. E., and Johnston, C. E. (2015). Traffic noise masks acoustic signals of freshwater stream fish. *Biol. Conserv.* 187, 27–33. doi: 10.1016/j.biocon.2015.04.004

Järvenpää, M., and Lindstrom, K. (2004). Water turbidity by algal blooms causes mating system breakdown in a shallow-water fish, the sand goby *Pomatoschistus minutus*. *Proc. Biol. Sci. B* 271, 2361–2365. doi: 10.1098/rspb.2004.2870

Klein, A., Münz, H., and Bleckmann, H. (2013). The functional significance of lateral line canal morphology on the trunk of the marine teleost *Xiphister atropurpureus* (Stichaeidae). *J. Comp. Physiol. A* 199, 735–749. doi: 10.1007/s00359-013-0834-6

Miller, C. W., and Svensson, E. I. (2014). Sexual selection in complex environments. *Annu. Rev.* 59, 427–445. doi: 10.1146/annurev-ento-011613-162044

Miller, P. J. (1986). "Gobiidae," in *Fishes of the North-Eastern Atlantic and the Mediterranean*, Vol. 3, ed P. J. P. Whitehead (Paris:UNESCO), 1019–1085.

Myrberg, A. J., and Lugli, M. (2006). "Reproductive behavior and acoustical interactions," in *Communication in Fishes*, Vol. 1, ed F. Ladich (Enfield, NH: Science Publishers), 149–176.

Nedelec, S. L., Radford, A. N., Pearl, L., Nedelec, B., McCormick, M. I., Meekan, M. G., et al. (2017). Motorboat noise impacts parental behavior and offspring survival in a reef fish. *Proc. Biol. Sci. B* 284:0170143. doi: 10.1098/rspb.2017.0143

Partan, S. R. (2017). Multimodal shifts in noise: switching channels to communicate through rapid environmental change. *Anim. Behav.* 124, 325–337. doi: 10.1016/j.anbehav.2016.08.003

Partan, S. R., Fulmer, A. G., Gounard, M. A., and Redmond, J. E. (2010). Multimodal alarm behavior in urban and rural gray squirrels studied by means of observation and a mechanical robot. *Curr. Zool.* 56, 313–326.

Pedroso, S. S., Bolgan, M., Jordão, J. M., Fonseca, P. J., and Amorim, M. C. P. (2012). "Acoustic communication in *Pomatoschistus* spp.: a comparison between closely related species," in *Advances in Experimental Medicine and Biology* (New York, NY: Springer), 113–115.

Picciulin, M., Sebastianutto, L., Codarin, A., Calcagno, G., and Ferrero, E. A. (2012). Brown meagre vocalization rate increases during repetitive boat noise exposures: a possible case of vocal compensation. *J. Acoust. Soc. Am.* 132, 3118–3124. doi: 10.1121/1.4756928

Picciulin, M., Sebastianutto, L., Codarin, A., Farina, A., and Ferrero, E. A. (2010). *In situ* behavioural responses to boat noise exposure of *Gobius cruentatus* (Gmelin, 1789, fam. Gobiidae) and *Chromis chromis* (Linnaeus, 1758, fam. Pomacentridae) living in a marine protected area. *J. Exp. Marine Biol. Ecol.* 386, 125–132. doi: 10.1016/j.jembe.2010.02.012

Popper, A. N., Hawkins, A. D., Fay, R. R., Mann, D., Bartol, S., Carlson, T., et al. (2014). *Sound Exposure Guidelines for Fishes and Sea Turtles: A Technical Report Prepared by ANSI-accredited Standards Committee S3/SC1 and Registered With ANSI. ASA S3/SC1.4 TR-2014, Springerbriefs in Oceanography*. Cham: Springer International Publishing; ASA Press.

Radford, A. N., Kerridge, E., and Simpson, S. D. (2014). Acoustic communication in a noisy world: can fish compete with anthropogenic noise? *Behav. Ecol.* 25, 1022–1030. doi: 10.1093/beheco/aru029

R Core Team (2017). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. Available online at: https://www.R-project.org/

Ricker, W. E. (1975). Computation and inter-pretation of biological statistics of fish populations. *Bull. of the Fish. Res. Board of Canada* 191, 1–382.

Rowe, C. (1999). Reciever psychology and the evolution of multicomponent signals. *Anim. Behav.* 58, 921–931. doi: 10.1006/anbe.1999.1242

Rowe, S., and Hutchings, J. A. (2006). Sound production by atlantic cod during spawning. *Trans. Am. Fish. Soc.* 135, 529–538. doi: 10.1577/T04-061.1

Slabbekoorn, H., Bouton, N., Van Opzeeland, I., Coers, A., Ten Cate, C., and Popper, A. (2010). A noisy spring: the impact of globally rising underwater sound levels on fish. *Trends Ecol. Evol.* 25, 419–427. doi: 10.1016/j.tree.2010.04.005

Stanley, J. A., Van Parijs, S. M., and Hatch, L. T. (2017). Underwater sound from vessel traffic reduces the effective communication range in Atlantic cod and haddock. *Sci. Rep.* 7:14633. doi: 10.1038/s41598-017-14743-9

Tuomainen, U., and Candolin, U. (2011). Behavioural responses to human-induced environmental change. *Biol. Rev.* 86, 640–657. doi: 10.1111/j.1469-185X.2010.00164.x

van der Sluijs, I., Gray, S. M., Amorim, M. C. P., Barber, I., Candolin, U., Hendry, A. P., et al. (2011). Communication in troubled waters: responses of fish communication systems to changing environments. *Evol. Ecol.* 25, 623–640. doi: 10.1007/s10682-010-9450-x

Vasconcelos, R. O., Amorim, M. C. P., and Ladich, F. (2007). Effects of ship noise on the detectability of communication signals in the Lusitanian toadfish. *J. Exp. Biol.* 210, 2104–2112. doi: 10.1242/jeb.004317

Venables, W. N., and Ripley, B. D. (2002). *Modern Applied Statistics With S*. New York, NY: Springer.

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Male Field Cricket Songs Are Altered After Aggressive Interactions

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To address the hypothesis that male acoustic sexual advertisement signals, in addition to chemical signals, might be indicators of aggressiveness, we examined the relationship between levels of aggression/dominance status and acoustic sexual advertisement signals in the field cricket *Gryllus integer*. Males were paired in aggression trials and recorded the night before and night after the trial. This allowed us to test whether aggression is inherently linked to song phenotypes, or whether aggressive interactions cause males to alter their songs. We found that dominant (winning) males signaled with higher energy, amplitude, and power the night after winning an aggressive encounter, but we could not detect any differences before the encounter. Time spent calling and the number of calling bouts were apparently unrelated to aggression, whereas winning males increased their bout lengths after winning, and losing males decreased their bout lengths after losing.

Keywords: communication, field cricket, acoustic signal, calling song, aggression, sexual selection, *Gryllus integer*

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INTRODUCTION

Sexual selection theory proposes that the selective sex (usually females) selects mates because of preferences for particular traits in the selected sex (usually males), allowing the traits to spread within a population even when those traits appear maladaptive (Darwin, 1874). Males will benefit from producing these traits if this increases their chances of mating (Fisher, 1930), while females will benefit from selecting mates based upon their preferred traits if the traits communicate some aspect of male quality that benefits the female directly (Price et al., 1993) or can benefit her offspring (Zahavi, 1977; Andersson, 1982; Hamilton and Zuk, 1982). Early signaling theory hypothesized that this link was maintained by "handicap signals:" sexual advertisement signals that are inextricably linked to male quality because they are costly to produce (thereby constraining them to be honest) and therefore can only be produced by high-quality males (Zahavi, 1975; Grafen, 1990). Later, Getty (2006) theorized that high-quality males do not "handicap" themselves by investing larger amounts of energy into their signals, but rather are simply more efficient at converting energy into signals, which reduces the cost of producing a large or extravagant signal. Another hypothesis states that it is not the cost of the signal, but rather the potential cost of cheating that keeps these signals honest (Számadó, 2011). According to this hypothesis, most males are able to produce a high-quality signal indicating, for example, that they are very large, but only large males are able to bear the cost of being challenged by other large males responding to their signals (Számadó, 2011).

One aspect of male quality that may be signaled to females is male aggression level. There are a few possible causes for a relationship between male sexual advertisement signals and aggressiveness. First, females may prefer aggressive males and select for males that signal their aggressiveness. In several species aggression levels are linked to reproductive success because females prefer or are constrained to mate with dominant males (Potter et al., 1976; Berglund and Rosenqvist, 2001; López et al., 2002; Double and Cockburn, 2003). Females that prefer aggressive males benefit directly (via access to better territories) or indirectly (any genetic components of aggression would be passed down to the female's offspring). Males that signal their aggressiveness would be at an advantage because females could select them without witnessing aggressive interactions. This type of signal would be constrained to honesty because genuinely aggressive males could challenge other males that dishonestly signal their aggressiveness, and losing those aggressive interactions would be extremely costly to dishonest signalers. Alternatively, females may simply prefer a trait that correlates with both aggression and acoustic signals. For example, acoustic signaling (Hoback and Wagner, 1997) and aggressive behaviors (Hack, 1995) are energetically costly, and only males in good condition (genetic/aerobic/energetic/body) can invest in acoustic signaling and aggression.

To address the hypothesis that male acoustic signals might be indicators of aggressiveness, we performed an experiment on the connection between levels of aggression and/or dominance status with acoustic sexual advertisement signals in the western stutter-trilling cricket, *Gryllus integer*. *Gryllus integer* males employ multi-modal courtship, using both chemical and acoustic signals to attract females. *Gryllus integer* females prefer the cuticular hydrocarbons of dominant males (Kortet and Hedrick, 2005); thus it may be possible that males are signaling their aggression acoustically as well.

Gryllus integer is an ideal species with which to study the relationship between acoustic signals and aggression, for two reasons. First, male *Gryllus integer* produce an acoustic signal to attract sexually receptive females. The signal is composed of a repeated series of chirps, strung together into bouts. Bouts are defined as periods of chirping with no interruptions longer than 0.1 s (Hedrick, 1986). Bout length is heritable (Hedrick, 1988), and females prefer males that produce longer bouts (Hedrick, 1986). Second, males that produce acoustic signals with longer bouts attract predators (Walker, 1964; Burk, 1982; Zuk and Kolluru, 1998), parasitoids (Cade, 1975; Wagner, 1996), and competitors (Gerhardt and Huber, 2002; Leonard and Hedrick, 2009; Jang, 2011; McCarthy et al., 2013) in addition to females. Competitor males may engage the signaling males in energetically taxing aggressive interactions (Hack, 1995; Tachon et al., 1999) or intercept and mate with females that are also attracted to the acoustic signals (Cade, 1980). If males attract competitor males via their acoustic signals, the males engage in energetically taxing aggressive behaviors with each other (Hack, 1995; Tachon et al., 1999). Thus, the most successful males will have an acoustic signal that is attractive to females while also maintaining aggression levels sufficient to defeat rival males. The question becomes: are male aggression levels and/or dominance statuses linked to acoustic signals, allowing females to gain more information about males from their songs?

Many studies have examined whether and how animal signals are used in aggressive contexts, but only a few have been able to show conclusively that those signals are communicating honest information about the signaler's aggressiveness (Searcy and Beecher, 2009). For example, researchers have established that low-amplitude song is an aggressive signal in the songbirds that use it (Akçay et al., 2015). In addition, Wagner (1992) discovered that frogs lower the carrier frequency of their calls as an honest signal of fighting ability.

Only a handful of studies have examined the direct relationship between aggressive behaviors and acoustic sexual signaling in crickets, with conflicting results. One study found a link between aggression and some aspects of signal structure and signaling effort (Bertram and Rook, 2012), specifically pulse length, pulses per chirp, chirp length, carrier frequency, and amplitude, while others found no link (Wilson et al., 2009; Fitzsimmons and Bertram, 2013). However, only one of these studies (Bertram and Rook, 2012) measured fine song parameters. Given the paucity of data on this relationship, particularly on fine song parameters, further experiments are required.

Acoustic sexual signals may be linked to aggression in two ways. Aggressive males' songs may be intrinsically different from the songs of less aggressive males, for example if both aggressiveness and signal quality covary with an aspect of male condition. Alternatively, aggressive interactions may alter male songs, such that males up- or down-regulate certain features of their acoustic signals depending upon the outcome of an aggressive interaction, allowing females to detect males that have won fights via their acoustic signals. (Whether these particular features are attractive or preferred by females requires further study).

We conducted an experiment using *Gryllus integer* males that were paired in aggression trials and recorded them the night before and after the trial. If aggression is linked to song phenotypes, then we predicted that males that displayed high levels of aggression in their aggression trials would have different songs than males that displayed low levels of aggression in their aggression trials, but each individual male's songs would not be significantly different pre- and post-trial. If aggressive interactions cause males to alter their songs, then we predicted that individual male songs would differ pre- and post-trial, depending upon the male's dominance status.

METHODS

Specimens

Juvenile *Gryllus integer* were raised from eggs laid by females caught from the field in Davis, CA in the summer of 2012 and reared in family boxes (32 cm L × 18 cm W × 12 cm H). Juveniles were sorted from family boxes into individual waxed paper cups when they were approximately one-quarter adult size. Crickets were provided with chick starter *ad libitum* and water. Crickets were kept physically isolated from one another but not acoustically isolated. Two weeks after completion of the adult molt, males ($n = 42$) were recorded overnight. The following day males were placed into their aggression trials ($n = 21$ dyads), and then recorded overnight again.

Aggression Trials

The day before aggression trials (pre-trial song recording), males were randomly paired into dyads. Males were marked with a single dot of Wite-Out$^{\text{TM}}$ on either the left or right side of the pronotum to allow for individual recognition during the trial. On the day of the trial, males were placed under clear plastic vials at the center of a small arena lined with sand (20 cm L × 20 cm W × 7 cm H) and allowed to acclimate for 2 min. After 2 min, the vials were removed, and males were allowed to interact for 6 min. All aggression trials were recorded using a Canon ZR500 camera on SONY Mini DV cassette tapes, and dominance was determined by watching tape playback. Aggressive behaviors were awarded points using an all-occurrence observation method based on an ethogram from Adamo and Hoy (1995), with more aggressive behaviors worth more points (antennal fencing = 1 point; kick = 2 points; mandible flare, lunge, chase, or bite = 3 points; grapple = 4 points). In 100% of the trials that escalated to grapples (7 of 21 total trials), the male with the higher aggressiveness score also won the most grapples during the trial. Therefore, in each dyad, males with higher aggressiveness scores at the end of the trial were determined to be "dominant" and those with lower scores "subordinate."

Song Recording

Males were placed inside a small plastic container (16 cm L × 18 cm W × 12 cm H) that was then placed inside another plastic tub (40 cm L × 27.5 cm W × 23 cm H) padded with sound-insulating foam with a closed top to prevent sound transfer between different containers. The containers were inside an acoustic chamber for recording, to reduce ambient noise in recordings. Recordings were made using a SM Pro Audi PR8E enhanced 8 channel preamp, Echo Audiofire12 interface and Behringer Super Cardioid XM 1800S microphones, with a 44,100 Hz sampling rate.

Songs were recorded overnight (∼17:30–10:00 the next day) using the computer program Reaper (Cockos, New York, NY, USA). In Reaper, song files were parsed to remove all silence and these smaller files were run through the amplitude detector in Raven Pro, version 1.5 (Cornell lab of Ornithology, Ithaca, NY, USA). We used a Hamming spectrogram view set to 42.7 ms, filtering out frequencies below 1,000 Hz and above 17,000 Hz. Most cricket songs are produced around 5 kHz (ranging between 2 and 8 kHz), so this filter did not affect data collection (Robillard et al., 2013). The amplitude detector settings were: 2,000 Hz, 0.21 s, 0.05 smoothing, which could accurately detect individual bouts. All detections were checked by hand to remove false positives and include false negatives. For each bout, the detector measured the value for various measures of the following parameters: frequency, amplitude, energy, power, and entropy. **Table 1** contains definitions of each of these parameters. Note that Raven measures amplitude using a custom unit, "U," that does not reflect absolute amplitude in dB, but rather the relative amplitude of different sounds. Thus, reported measures should be considered relative to one another and not as absolute amplitude. While amplitude is notoriously difficult to accurately measure, our set up accounts for many of these difficulties by limiting cricket movement to a small area (without tethering the animal to a single location, influencing its behavior) and orienting the microphone above the cricket. Previous work using this set up with males not subjected to behavioral trials or other disruption across three consecutive nights of recording showed significant differences in amplitude between individuals, but could not detect significant differences across nights within individuals (repeatability = 0.514, $p = 0.0007$, Bunting, unpublished data). In addition to these parameters, the Raven selections allowed us to calculate the total time spent calling, the total number of bouts, and the mean bout length for each night.

Data Analysis

All statistical analysis was performed using general linear models (GLM) in R statistical software package, R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria). Mixed models were used for all analyses with dyad number as a random effect because aggressive behaviors within a dyad are not independent. Models were compared to one another using Akaike Information Criteria (AIC), and Akaike weights (the probability that one model is a better fit for a particular set of data; Anderson, 2008) were calculated (w_i).

To test the hypothesis that dominant and subordinate males have intrinsically different songs, pre-trial values of each song parameter were used as outcome variables. If aggression trial results predicted these outcome variables better than post-trial values of these variables, it would be evidence that aggression and acoustic signals are intrinsically linked, since these recordings took place before any males experienced aggressive interactions. To test the hypothesis that male songs are altered by aggressive interactions, post-trial values of each song parameter were used as outcome variables. If aggression trial results predicted these outcome variables better than pre-trial values of these variables, it would be evidence that aggressive interactions alter acoustic signals. Each model set contained two null models: a standard null model with no covariates, and an experimental null model where the only covariate was the outcome variable on the opposite night (i.e., when the pre-trial value is the outcome variable, the post-trial value is in the experimental null model and vice versa). If the experimental null model carried the most weight in any of the model sets, then that would be evidence that acoustic signals are not covarying or changing with aggression levels and/or experience, but rather are intrinsic to the individual and/or not linked to aggression. It is important to note that these hypotheses are not necessarily mutually exclusive: aggressive experience and intrinsic individual differences may both influence acoustic advertisement signals. However, this model selection process allows us to assess the relative influence of these factors on song phenotype and determine which of these has a larger effect on each song parameter.

All model sets included models with single covariates: aggressiveness score, difference in aggressiveness score (each male's aggressiveness score minus his opponent's score), dominance status (dominant or subordinate), male weight,

TABLE 1 | Pre-trial models of signaling effort and fine song parameters.

	Definition	Model family	Highest ranked model	Weight	Fixed effects	*P*	Mean estimate	Confidence interval	
								2.50%	97.50%
Signaled	Signaled at least once during recording period (Yes/No)	Binomial	Dominance status	0.776	Dominant (Intercept)	0.0000314	0.99	0.9968	0.999
					Subordinate	0.00099	0.99	0.9977	1
Time spent Signaling	Total time spent signaling during recording Period (Units: s)	Gaussian	Dominance status	0.21	Dominant (Intercept)	0.387	52.98	−284.89	390.86
					Subordinate	0.0843	408.98	−68.85	886.81
Total bouts Produced	Total bouts produced during recording period	Gaussian	Dominance status	0.204	Dominant (Intercept)	0.3942	29.67	−172.76	232.1
					Subordinate	0.0792	252.81	−33.47	539.09
Bout length	Mean length of signal that contained no silence longer than 0.1 s (Units: s)	Gaussian	Experimental null	0.96	Intercept	0.03349	0.69	0.15	1.23
					N2 Bout Length	0.006658	0.58	0.31	0.85
Average Amplitude	Mean amplitude value within a bout (Units: U).	Gaussian	Weight	0.21	Intercept	0.1039	−0.18	−0.41	0.05
					Weight	0.0945	0.57	−0.14	1.27
Maximum Amplitude	The maximum of all amplitude values within a bout (Units: U).	Gaussian	Experimental null	0.771	Intercept	0.0563	326717.51	10796.37	642638.7
					N2 Max Amp	0.0044	0.78	0.46	1.1
Aggregate entropy	Measure of distribution of energy across frequencies in sound. Higher values indicate more frequencies in sound (Unitless).	Gaussian	Experimental null	0.4	Intercept	0.010934	3.16	1.46	4.86
					N2 Agg Entropy	0.00222	0.47	0.22	0.73
Average entropy	Mean level of entropy in the bout. Measure of distribution of energy across frequencies in sound. Higher values indicate more frequencies in sound (Unitless).	Gaussian	Dominance score*N2	0.57	Intercept	0.0041	5.25	2.6	7.91
					Dominance Score	0.0325	−0.09	−0.17	−0.01
					N2 Avg Entropy	0.1581	0.21	−0.17	0.6
					Dominance Score*N2	0.0268	0.01	0	0.03
Average power	The average of the energy per unit time in the bout (Units: dB).	Gaussian	Experimental null	0.678	Intercept	0.0545	28.89	1.36	58.39
					N2 Avg Power	0.0000662	0.72	0.43	1.01
Maximum power	The highest energy per unit time in the bout (Units: dB re 1 dimensionless sample unit).	Gaussian	Dominance status*N2	0.55	Dominant (Intercept)	0.0588	40.69	−2.98	84.37
					Subordinate	0.002	107.07	60.52	153.61
					N2 Max Power	0.0032	0.69	0.36	1.03
					SUB*N2 Max Power	0.0021	−0.81	−1.16	−0.46
Center frequency	The frequency that divides the selection into two frequency intervals of equal energy (Units: Hz).	Gaussian	Experimental null	0.79	Intercept	0.004381	3480.69	2054.19	4907.2
					N2 Center Freq	0.0594	0.29	0	0.57
Maximum frequency	The frequency at which maximum power occurs within the bout (Units: Hz).	Gaussian	Experimental null	0.77	Intercept	0.0039	2712.26	1638.05	3786.48
					M2 Max Freq	0.009183	0.43	0.21	0.64
Energy	The total energy within the bout (Units: dB).	Gaussian	Experimental null	0.78	Intercept	0.0509	43.95	3.25	84.65
					N2 Energy	0.0039	0.71	0.43	0.99

difference in weight (each male's weight minus his opponent's weight), the number of grapples (fights), and the number of grapples won (wins). Where appropriate, combinations of these covariates were used in larger model sets to determine the effect of interactions between these variables. All models used a Gaussian link function, with the exception of models testing whether each male signaled at least once on either night, which used a binomial link function.

RESULTS

All results are summarized in **Table 1** (Pre-trial) and **Table 2** (Post-trial), each of which includes all song structures and definitions, the model that carried the most weight in each model set, significance levels, mean estimates, and confidence intervals. It is important to note that negative results could be due to Type II errors, as our sample sizes were small.

Signaling Effort

Pre-trial

No significant differences were detected between dominant and subordinate males in either the time spent signaling (**Figure 1**) or the number of bouts they produced pre-trial. The best model in both sets contained dominance status as a covariate, but weight was low ($w_i = 0.21$ for time, $w_i = 0.20$ for bouts) and the effect was not significant. There was also no difference in the bout length produced by dominant or subordinate males (**Figure 2**), though the mean for dominant males was larger than subordinate males); the experimental null model carried the most weight ($w_i = 0.80$) in that model set, with a significant effect of post-trial bout length. However, dominant males were significantly less likely to signal at least once pre-trial (11/21 dominant males vs. 18/21 subordinate males signaled at least once). The best model contained dominance status as the only covariate and carried a significant proportion of the weight ($w_i = 0.78$), with a significant effect of dominance status.

Post-trial

After the trial, similar to the pre-trial period, there were no significant differences in time spent signaling (**Figure 1**, null model, $w_i = 0.70$) or the number of bouts produced (null model, $w_i = 0.63$). The pre-trial difference in likelihood to signal at least once over the course of the night was eliminated post-trial (13/21 dominant males and 14/21 subordinate males signaled at least once). There was a significant effect of having signaled pre-trial, and a model containing only pre-trial caller status as a covariate carried the most weight ($w_i = 0.61$). For bout length, a model containing an interaction of dominance status and pre-trial bout length carried the most weight ($w_i = 0.56$), with a statistical trend of dominance status, plus significant effects of pre-trial bout length, and a significant interaction that indicates males increase their bout lengths after an aggressive interaction (**Figure 2**). While subordinate males also increase their bout lengths after an aggressive interaction, the margin of increase was smaller for subordinate males than dominant males.

Fine Song Parameters

Pre-trial

An experimental model best explained the pre-trial values of two fine song parameters. Dominant males signaled with significantly lower maximum power (**Figure 3**). The best-supported model contained a significant interaction of dominance status and post-trial values ($w_i = 0.55$). Average entropy was best explained with a model containing an interaction between post-trial entropy and aggressiveness score, indicating that more aggressive males called with higher levels of entropy ($w_i = 0.57$). Pre-trial values for aggregate entropy ($w_i = 0.40$), average power ($w_i = 0.68$), center frequency ($w_i = 0.79$), energy ($w_i = 0.78$), maximum amplitude ($w_i = 0.77$), and maximum frequency ($w_i = 0.77$) were best explained by the experimental null model with significant effects of post-trial values, indicating that these measures do not primarily signal dominance as an intrinsic feature of the songs of highly aggressive males. Average amplitude (**Figure 4**) was equally well explained by the true null model and model containing only male weight ($w_i = 0.21$). Larger males signaled more loudly, but the relationship was not statistically significant.

Post-trial

There were more significant results for post-trial fine song parameters than for pre-trial fine song parameters. A model containing an interaction between pre-trial values and aggressiveness score carried the most weight for model sets explaining maximum amplitude ($w_i = 0.99$) and maximum power ($w_i = 0.53$), indicating that highly aggressive males increased these amplitude measures after aggressive encounters. Males with higher aggressiveness scores signaled with higher amplitude and power after an aggressive encounter but signaled with lower amplitude and power before an aggressive encounter (**Figures 3**, **4**). For maximum power (**Figure 3**), there were significant effects of aggressiveness score, pre-trial value, and the interaction between the two. For maximum amplitude (**Figure 4**), there was a near-significant effect of aggressiveness score and a significant interaction between aggressiveness score and pre-trial maximum amplitude, but no significant effect of pre-trial values alone. A model containing an interaction between dominance status and pre-trial song energy carried the most weight ($w_i = 0.51$), with significant effects of pre-trial values and significant interaction, indicating dominant males signaled with higher energy after an aggressive interaction but not before (**Figure 5**). Males with more positive differences in aggression score (i.e., males that displayed higher numbers of aggressive behaviors relative to their opponent) signaled with higher average power, while the best-supported model likewise contained an interaction between difference in aggression score and pre-trial power ($w_i = 0.93$), with significant effects of pre-trial power and interaction. Post-trial aggregate entropy ($w_i = 0.76$), average entropy ($w_i = 0.50$), center frequency ($w_i = 0.77$), and maximum frequency ($w_i = 0.77$) were once again best explained by the experimental null model, while post-trial average amplitude was best explained with the standard null model ($w_i = 0.61$), with male weight once again positively correlating with amplitude, though not significantly.

TABLE 2 | Post-trial models of signaling effort and fine song parameters.

Variable	Definition	Model family	Highest ranked model	Weight	Fixed effects	*P*	Mean	Confidence interval	
							Estimate	2.50%	97.50%
Signaled	Signaled at least once during recording period (Yes/No)	Binomial	Signaled N1	0.61	No Signal N1 (Intercept)	0.067	0.2314	0.0766	0.5225
					Signaled N1	0.0007	0.941	0.7613	0.9876
Time spent signaling	Total time spent signaling during recording period (Units: s)	Gaussian	Null	0.696	Intercept	0.079127	162.61	−7.84	333.07
Total bouts produced	Total bouts produced during recording period	Gaussian	Null	0.634	Intercept	0.0755	100.83	−2.29	203.95
Bout length	Mean length of signal that contained no silence longer than 0.1 s (Units: s)	Gaussian	Dominance status*N1	0.562	Dominant (Intercept)	0.187	−0.65	−1.97	0.68
					Subordinate	0.06	1.38	0.02	2.75
					N1 Bout Length	0.0028	1.44	0.83	2.05
					Sub*N1 Bout Length	0.0293	−0.92	−1.62	−0.21
Average Amplitude	Mean amplitude value within a bout (Units: U).	Gaussian	Null	0.61	Intercept	0.3213	−0.01	−0.05	0.03
Maximum Amplitude	The maximum of all amplitude values within a bout (Units: U).	Gaussian	Dominance score*N1	0.99	Intercept	4.80E−03	561981.33	26206.08	855756.58
					Dominance Score	0.07	−10233.31	−19895.44	−571.17
					N1 Max Amp	0.522	0.09	−0.15	−0.33
					Dominance Score*N1	0.000133	0.02	0.01	0.02
Aggregate Entropy	Measure of distribution of energy across frequencies in sound. Higher values indicate more frequencies in sound (Unitless).	Gaussian	Experimental null	0.763	Intercept	0.1253	1.77	−0.82	4.36
					N1 Agg Entropy	0.0022	0.74	0.34	1.15
Average entropy	Mean level of entropy in the bout. Measure of distribution of energy across frequencies in sound. Higher values indicate more frequencies in sound (Unitless).	Gaussian	Experimental null	0.5	Intercept	0.260689	0.86	−1.54	3.26
					N1 Avg Entropy	0.000182	0.89	0.55	1.22
Average power	The average of the energy per unit time in the bout (Units: dB).	Gaussian	Score difference*N1	0.932	Intercept	2.61E−02	28.88	5.46	52.31
					Score Difference	0.005	−1.71	−2.74	−0.69
					N1 Avg Power	2.59E−05	0.69	0.46	0.93
					Score Difference*N1	0.00491	0.02	0.01	0.03
Maximum power	The highest energy per unit time in the bout (Units: dB re 1 dimensionless sample unit).	Gaussian	Dominance Score*N1	0.529	Intercept	0.0028	406.14	217.13	594.14
					Dominance Score	0.009	−9.45	−15.2	−3.71
					N1 Max Power	0.0145	−2.08	−3.51	−0.65
					Dominance Score*N1	0.009	0.07	0.03	0.11
Center frequency	The frequency that divides the selection into two frequency intervals of equal energy (Units: Hz).	Gaussian	Experimental null	0.773	Intercept	0.0711	2406.02	−179.52	4991.55
					N1 Center Freq	0.0594	0.53	0.01	1.06
Maximum frequency	The frequency at which maximum power occurs within the bout (Units: Hz).	Gaussian	Experimental null	0.766	Intercept	0.372	404.26	−1873.62	2682.14
					N1 Max Freq	0.000736	0.94	0.49	1.39
Energy	The total energy within the bout (Units: dB).	Gaussian	Dominance status*N1	0.51	Dominant (Intercept)	1.80E−01	−26.56	−79.23	26.11
					Subordinate	0.0186	111.58	29.56	193.6
					N1 Energy	3.00E−04	1.18	0.82	1.54
					Sub*N1 Energy	0.0169	−0.78	−1.34	−0.22

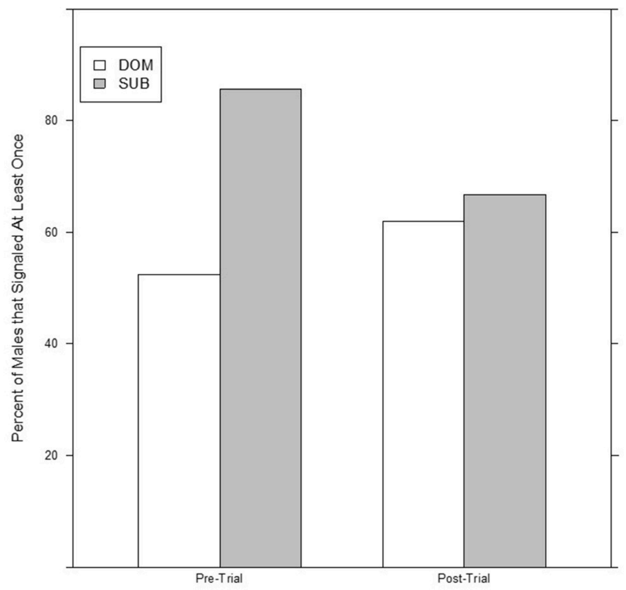

FIGURE 1 | Dominant males are significantly less likely to signal the night before an aggressive interaction, but there is no difference between dominant and subordinate males after an aggressive interaction.

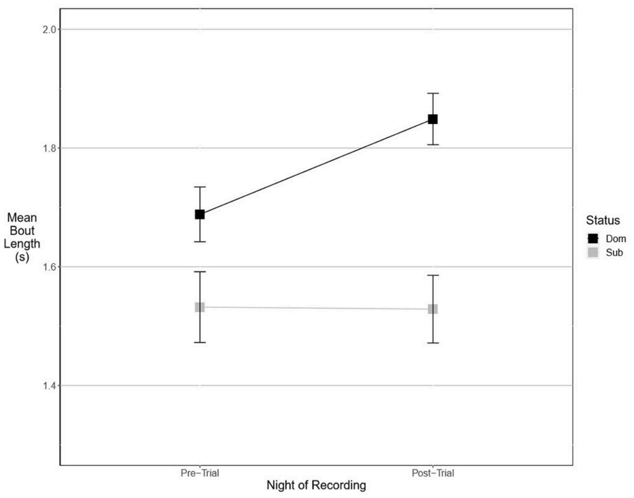

FIGURE 2 | Dominant males signal with longer bouts than subordinate males. This margin is larger after an aggressive interaction. Graph of mean estimates of model: Bout Length ∼ (1|ID) + (1|Pair) + Score Result *Night of Recording.

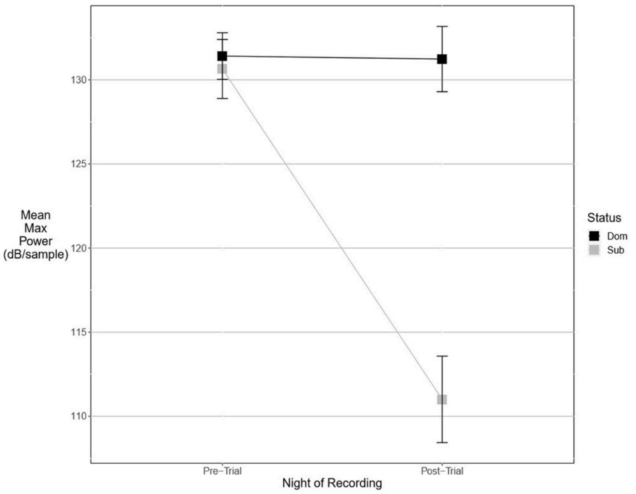

FIGURE 3 | Dominant males signal with higher power only after an aggressive interaction. Graph of mean estimates of model: Power ∼ (1|ID) + (1|Pair) + Score Result *Night of Recording.

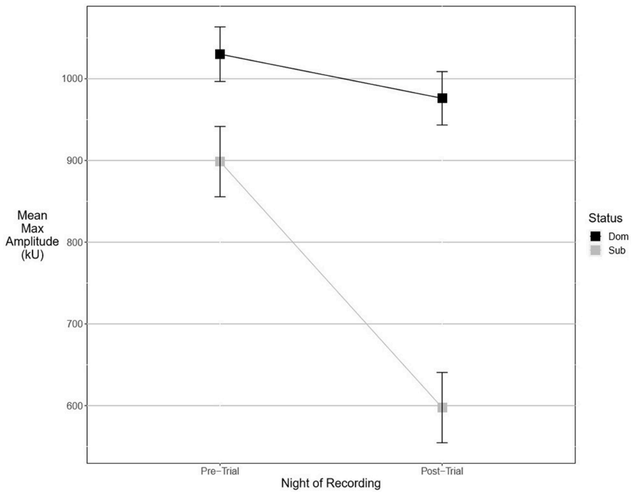

FIGURE 4 | Dominant males signal with higher amplitude than subordinate males. This margin is significant after an aggressive interaction. Graph of mean estimates of model: Amplitude ∼ (1|ID) + (1|Pair) + Score Result *Night of Recording.

DISCUSSION

We tested two alternative hypotheses for how male acoustic signals were related to aggression: (1) that more aggressive and less aggressive males had intrinsically different signals, or (2) that the signals would change depending upon the result of an aggressive interaction. While these hypotheses are not necessarily mutually exclusive and we found some evidence for both, we found stronger support for the latter hypothesis. A model containing an aggression variable best predicted two pre-trial values of fine song parameters, but best predicted six post-trial values. Dominant males signaled with higher energy, amplitude, and power than subordinate males the night after winning an aggressive encounter but did not before the trial, for this sample of crickets. A model that contained their pre-trial values best predicted nearly all of the fine song parameters measured, even those that changed significantly after aggression trials. This indicates that these measures are highly repeatable within individuals, and the individual's genetic signal phenotype and/or long-lasting early environmental effects limit any effect aggressive experience can have on these measures. Interestingly,

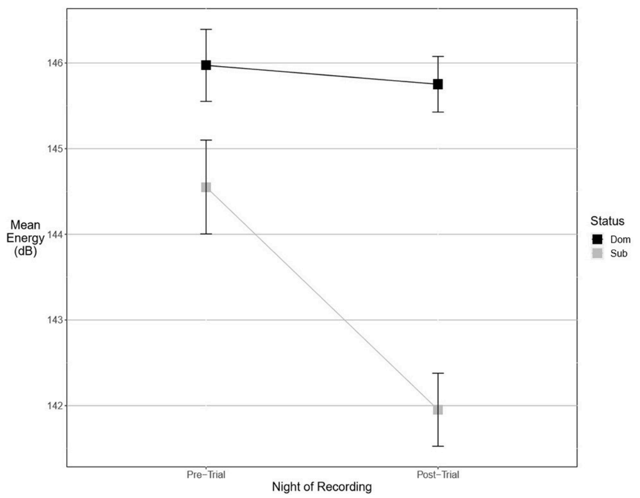

FIGURE 5 | Dominant males signal with higher energy than subordinate males. This margin is significant after an aggressive interaction. Graph of mean estimates of model: Energy ∼ (1|ID) + (1|Pair) + Score Result *Night of Recording.

aggressive interactions do not apparently change the chemical signals of these crickets: females prefer the scent of dominant males before the males have ever been in a fight (Kortet and Hedrick, 2005).

Although our sample size was relatively low, our results were consistent with previous work indicating that only certain aspects of acoustic signaling effort are significantly linked with aggression in crickets. Wilson et al. (2009) recorded lab-raised *Acheta domesticus* for four consecutive nights and found no correlation between signaling effort (time spent calling, the number of bouts produced, and bout length) and aggression, although they did not examine how signaling changed with aggressive interactions, and thus only recorded male songs before aggression trials. Bertram and Rook (2012) recorded lab-raised *Gryllus assimilis* for 14 days and also found no correlation between time spent signaling and aggression. They did, however, find that aggression was correlated with pulse length, pulses per chirp, chirp length, carrier frequency, and amplitude. Fitzsimmons and Bertram (2013) used lab-raised and field-caught *Gryllus veletis* to test the relationship between acoustic signals and aggression. They examined how song effort differed before and after a fight and found no difference between pre-fight signaling effort and post-fight signaling effort in either dominant or subordinate males (Fitzsimmons and Bertram, 2013).

In our study, the time spent calling and the number of bouts produced were not correlated with aggression, nor did they change significantly after an aggressive interaction. These aspects of male signaling effort may be signaling some aspect of quality—either genetic or current body condition—that does not influence or is uncorrelated with aggressiveness (or influences signaling effort to a much higher degree than aggressiveness). Alternatively, investment in signaling may be subject to energetic trade-offs as it is in *Gryllus texensis* (Bertram and Warren, 2005), such that increasing signaling effort was not possible for either dominant or subordinate males, particularly after a potentially energetically draining aggressive interaction. Similar to Bertram and Rook (2012), we found a positive relationship between dominance and bout length, but our statistical methods also allowed us to discover an alteration of bout length influenced by aggressive interactions. Dominant males increased their (already longer) bout lengths after winning, while subordinate males decreased their (already shorter) bout lengths after losing. Females prefer males that call with longer bouts in this species (Hedrick, 1986), thus females may be selecting for males that win fights by preferring longer bouts.

There was a significant difference between dominant and subordinate males in whether they signaled the night before the trial (18/21 of subordinate males signaled vs. only 11 of 21 dominant males). This difference disappeared post-trial (14/21 subordinate males vs. 13/21 dominant males), which could indicate, based on these numbers, that dominant males are increasing their signaling, while subordinate males are decreasing their signaling due to the result of the aggressive trial. Subordinate males may reduce their chances of encountering another male by not producing a signal that may attract them (Andersson, 1994; Gerhardt and Huber, 2002; Leonard and Hedrick, 2009; Jang, 2011), while dominant males may not benefit from such a decrease in signaling. However, examination of the raw numbers suggests the effect size is small with two new dominant signalers (out of 21) and four subordinate signalers (out of 21) ceasing signaling after encounters. While our small sample size increases the potential for Type II error and the evidence for a change in probability of signaling post-trial is weak, there is stronger support for the finding that dominant males are less likely to signal acoustically overall.

In our relatively limited sample, aggressive males signaled with higher amplitude, power, and energy than subordinate males in their acoustic signals after an aggressive interaction, but not before. Higher amplitude signals that contain more energy are going to transmit longer distances from the signaler, increasing the number of potential receivers. These high-amplitude signals will potentially attract more females, but also rival males (Gerhardt and Huber, 2002; Leonard and Hedrick, 2009; Jang, 2011; McCarthy et al., 2013). Winners of aggressive contests may perceive the risk of attracting rivals as reduced because of their victory. Alternatively, dominant males may be adjusting their signals because of alterations to their social environment. Patricelli and Krakauer (2010) discovered that male greater sage grouse (*Centrocercus urophasianus*) that are successful in mating can increase their signal investment when receivers are nearby, whereas males that do not mate successfully are not able to increase investment. Because these crickets were raised in physical isolation, the experimental aggressive interaction represents a dramatically changed social environment and dominant males could be adjusting their signals accordingly, while subordinate males are not able to adjust their signal.

In our analyses, the fine song parameters of average entropy, center frequency, and maximum frequency were not significantly different pre- or post-trial for either subordinate or dominant males. A likely reason these particular measures are not correlated with aggressiveness either pre- or post- trial is that the information contained in these measures may be (relatively) unrelated to aggression. These fine song parameters may be signaling species identification information, which necessarily must be minimally variable between males to avoid hybridization with other closely related species (Jaiswara et al., 2013; Bastian and Jacobs, 2015). Alternatively, the neural mechanisms of females may be tuned to a very narrow frequency distribution, providing no advantage to (and strong selection against) any frequency alterations. In *Teleogryllus oceanicus,* female auditory neural mechanisms are particularly tuned to a few very specific frequency ranges, one of which includes the mean peak frequency of male *T. oceanicus* acoustic signals (Pollack and Faulkes, 1998). Changing the frequency of a signal would be disadvantageous if females will not respond to the new frequency.

Models including the number of wins and/or the number of grapples never ranked among the top models for any outcome variable, despite the fact that these data are measures of the most aggressive interactions in this species. This is perhaps because aggression behaviors escalated to grapples in only a small proportion of dyads (7/21), reducing statistical power and increasing the risk of Type II error. Alternatively, it may be evidence that the effect of an aggressive interaction is less dependent upon the level of aggression that occurred within the interaction, but rather on the fact the interaction occurred at all: perhaps the degree of dominance matters less than simply being dominant, even by a small margin. This could explain why a relatively small proportion of dyads escalated to grapples; the cost of the grapple may be too high, if the reward does not proportionately increase.

One open question concerns the duration of the changes we observed in male signals. Because we only recorded males the night before and night after aggressive trials, we are unable to determine whether alterations of male signals are long-lasting, and whether these alterations are of different durations for subordinate vs. dominant males. Moreover, an imperative next step in studies such as this is to assess how these song differences and/or alterations influence female choice. Dominant males increase their bout lengths after an aggressive contest, and longer bout lengths are preferred by females (Hedrick, 1986), but there is less known about female preferences for fine song parameters in this species. Whereas dominant males have higher power, amplitude, and energy in their songs after an aggressive encounter, this does not mean that females are paying attention to those parameters of the signal or making mating decisions based on them. A female choice playback experiment varying those parameters of the song, while keeping other signal features constant, is needed to ascertain whether these changes are independently important in a mating context. Even if females are not using these factors to make mating decisions, these song alterations likely play a role in male-male competition. For example, potential competitors may avoid signals with higher power or amplitude, to reduce damaging aggressive interactions. Though highly consistent within individuals, acoustic signal bout length, power, amplitude, and energy do change due to aggressive experience, indicating that field crickets may be adjusting their signals based upon their social condition. Plasticity in signal phenotype or investment may allow crickets to signal more about themselves than just genetic background or body condition.

DATASET AVAILABILITY

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

AUTHOR CONTRIBUTIONS

JB and AH contributed conception and design of the study. JB collected the data and performed the statistical analysis. JB wrote the first draft of the manuscript. AH revised the manuscript and prepared it for publication. All authors read and approved the submitted version.

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REFERENCES

Adamo, S. A., and Hoy, R. R. (1995). Agonistic behaviour in male and female field crickets, *Gryllus bimaculatus*, and how behavioural context influences its expression. *Anim. Behav.* 49, 1491–1501. doi: 10.1016/0003-3472(95)90070-5

Akçay, Ç., Anderson, R. C., Nowicki, S., Beecher, M. D., and Searcy, W. A. (2015). Quiet threats: soft song as an aggressive signal in birds. *Anim. Behav.* 105, 267–274. doi: 10.1016/j.anbehav.2015.03.009

Anderson, D. R. (2008). *Model-Based Inference in the Life Sciences.* New York, NY: Springer. doi: 10.1007/978-0-387-74075-1

Andersson, M. (1982). Sexual selection, natural selection and quality advertisement. *Biol. J. Linn. Soc.* 17, 375–393. doi: 10.1111/j.1095-8312.1982.tb02028.x

Andersson, M. B. (1994). *Sexual Selection*. Princeton, NJ: Princeton University Press.

Bastian, A., and Jacobs, D. S. (2015). Listening carefully: increased perceptual acuity for species discrimination in multispecies signalling assemblages. *Anim. Behav.* 101, 141–154. doi: 10.1016/j.anbehav.2014.12.010

Berglund, A., and Rosenqvist, G. (2001). Male pipefish prefer dominant over attractive females. *Behav. Ecol.* 12, 402–406. doi: 10.1093/beheco/12.4.402

Bertram, S. M., and Rook, V. (2012). Relationship between condition, aggression, signaling, courtship, and egg laying in the field cricket, *Gryllus assimilis*. *Ethology* 118, 360–372. doi: 10.1111/j.1439-0310.2011.02019.x

Bertram, S. M., and Warren, P. S. (2005). Trade-offs in signaling components differ with signaling effort. *Anim. Behav.* 70, 477–484. doi: 10.1016/j.anbehav.2004.09.024

Burk, T. (1982). Evolutionary significance of predation on sexually signaling males. *Florida Entomol.* 65:90. doi: 10.2307/3494148

Cade, W. (1975). Acoustically orienting parasitoids: fly phonotaxis to cricket song. *Science* 190, 1312–1313. doi: 10.1126/science.190.4221.1312

Cade, W. (1980). Alternative male reproductive behaviors. *Florida Entomol.* 63, 30–45. doi: 10.2307/3494654

Darwin, C. (1874). *The Descent of Man and Selection in Relation to Sex, 2nd Edn*. London: John Murray.

Double, M. C., and Cockburn, A. (2003). Subordinate superb fairy-wrens (*Malurus cyaneus*) parasitize the reproductive success of attractive dominant males. *Proc. R. Soc. B Biol. Sci.* 270, 379–384. doi: 10.1098/rspb.2002.2261

Fisher, R. A. (1930). *The Genetical Theory of Natural Selection*. Oxford: Clarendon Press.

Fitzsimmons, L. P., and Bertram, S. M. (2013). Signaling effort does not predict aggressiveness in male spring field crickets. *Behav. Ecol. Sociobiol.* 67, 213–220. doi: 10.1007/s00265-012-1441-1

Gerhardt, H. C., and Huber, F. (2002). *Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions*. Chicago, IL: The University of Chicago Press.

Getty, T. (2006). Sexually selected signals are not similar to sports handicaps. *Trends Ecol. Evol.* 21, 83–88. doi: 10.1016/j.tree.2005.10.016

Grafen, A. (1990). Biological signals as handicaps. *J. Theor. Biol.* 144, 517–546. doi: 10.1016/S0022-5193(05)80088-8

Hack, M. A. (1995). The energetic costs of fighting in the house cricket, *Acheta domesticus* L. *Behav. Ecol.* 8, 28–36. doi: 10.1093/beheco/8.1.28

Hamilton, W. D., and Zuk, M. (1982). Heritable true fitness and bright birds: a role for parasites? *Science* 218, 384–387. doi: 10.1126/science.7123238

Hedrick, A. V. (1986). Female preferences for male calling bout duration in a field cricket. *Behav. Ecol. Sociobiol.* 19, 73–77. doi: 10.1007/BF00303845

Hedrick, A. V. (1988). Female choice and the heritability of attractive male traits: an empirical study. *Am. Nat.* 132, 267–276. doi: 10.1086/284849

Hoback, W. W., and Wagner, W. (1997). The energetic cost of calling in the variable field cricket, *Gryllus lineaticeps*. *Physiol. Entomol.* 22, 286–290. doi: 10.1111/j.1365-3032.1997.tb01170.x

Jaiswara, R., Nandi, D., and Balakrishnan, R. (2013). Examining the effectiveness of discriminant function analysis and cluster analysis in species identification of male field crickets based on their calling songs. *PLoS ONE* 8:e75930. doi: 10.1371/journal.pone.0075930

Jang, Y. (2011). Male responses to conspecific advertisement signals in the field cricket *Gryllus rubens* (*Orthoptera: Gryllidae*). *PLoS ONE* 6:e16063–e16068. doi: 10.1371/journal.pone.0016063

Kortet, R., and Hedrick, A. (2005). The scent of dominance: female field crickets use odour to predict the outcome of male competition. *Behav. Ecol. Sociobiol.* 59, 77–83. doi: 10.1007/s00265-005-0011-1

Leonard, A. S., and Hedrick, A. V. (2009). Male and female crickets use different decision rules in response to mating signals. *Behav. Ecol.* 20, 1175–1184. doi: 10.1093/beheco/arp115

López, P., Muñoz, A., and Martín, J. (2002). Symmetry, male dominance and female mate preferences in the Iberian rock lizard, *Lacerta monticola*. *Behav. Ecol. Sociobiol.* 52, 342–347. doi: 10.1007/s00265-002-0514-y

McCarthy, T. M., Keyes, J., and Cade, W. H. (2013). Phonotactic behavior of male field crickets (*Gryllus texensis*) in response to acoustic calls from conspecific males. *J. Insect Behav.* 26, 634–648. doi: 10.1007/s10905-013-9375-7

Patricelli, G. L., and Krakauer, A. H. (2010). Tactical allocation of effort among multiple signals in sage grouse: an experiment with a robotic female. *Behav. Ecol.* 21, 97–106. doi: 10.1093/beheco/arp155

Pollack, G. S., and Faulkes, Z. (1998). Representation of behaviorally relevant sound frequencies by auditory receptors in the cricket *Teleogryllus oceanicus*. *J. Exp. Biol.* 201, 155–163.

Potter, D. A., Wrensch, D. L., and Johnston, D. E. (1976). Aggression and mating success in male spider mites. *Science* 193, 160–161. doi: 10.1126/science.193.4248.160

Price, T., Schluter, D., and Heckman, N. E. (1993). Sexual selection when the female directly benefits. *Biol. J. Linn. Soc.* 48, 187–211. doi: 10.1111/j.1095-8312.1993.tb00887.x

Robillard, T., Montealegre-Z, F., Desutter-Grandcolas, L., Grandcolas, P., and Robert, D. (2013). Mechanisms of high-frequency song generation in brachypterous crickets and the role of ghost frequencies. *J. Exp. Biol.* 216, 2001–2011. doi: 10.1242/jeb.083964

Searcy, W. A., and Beecher, M. D. (2009). Song as an aggressive signal in songbirds. *Anim. Behav.* 78, 1281–1292. doi: 10.1016/j.anbehav.2009.08.011

Számadó, S. (2011). The cost of honesty and the fallacy of the handicap principle. *Anim. Behav.* 81, 3–10. doi: 10.1016/j.anbehav.2010.08.022

Tachon, G., Murray, A. M., Gray, D. A., and Cade, W. H. (1999). Agonistic displays and the benefits of fighting in the field cricket, *Gryllus bimaculatus*. *J. Insect Behav.* 12, 533–543. doi: 10.1023/A:1020970908541

Wagner, W. E. (1992). Deceptive or honest signaling of fighting ability? A test of alternative hypotheses for the function of changes in call dominant frequency by male cricket frogs. *Anim. Behav.* 44, 449–462.

Wagner, W. E. (1996). Convergent song preferences between female field crickets and acoustically orienting parasitoid flies. *Behav. Ecol.* 7, 279–285. doi: 10.1093/beheco/7.3.279

Walker, T. J. (1964). Experimental demonstration of a cat locating orthopteran prey by the prey's calling song. *Florida Entomol.* 47, 163–165.

Wilson, A. D. M., Whattam, E. M., Bennett, R., Visanuvimol, L., Lauzon, C., and Bertram, S. M. (2009). Behavioral correlations across activity, mating, exploration, aggression, and antipredator contexts in the European house cricket, *Acheta domesticus*. *Behav. Ecol. Sociobiol.* 64, 703–715. doi: 10.1007/s00265-009-0888-1

Zahavi, A. (1975). Mate selection—a selection for a handicap. *J. Theor. Biol.* 53, 205–214. doi: 10.1016/0022-5193(75)90111-3

Zahavi, A. (1977). The cost of honesty (further remarks on the handicap principle). *J. Theor. Biol.* 67:603. doi: 10.1016/0022-5193(77)90061-3

Zuk, M., and Kolluru, G. R. (1998). Exploitation of sexual signals by predators and parasitoids. *Q. Rev. Biol.* 73, 415–438. doi: 10.1086/420412

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Response Mode Choice in a Multimodally Duetting Paleotropical Pseudophylline Bushcricket

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Females of the pseudophylline bushcricket species *Onomarchus uninotatus* respond to a conspecific acoustic call with bouts of tremulation, followed by phonotaxis in some cases. This tremulation sends out a vibratory signal that propagates along the branch of the jackfruit trees where these animals are almost always found, and the male is able to localize the signal and perform vibrotaxis toward the female. Males are unable to localize the signal if it emanates from a branch unconnected to their perch, and therefore, female tremulation might not be a productive response when the nearest male is on an adjacent, disconnected tree. We hypothesized that female behavioral response choice between tremulation and phonotaxis might vary with distance from the caller. A semi-naturalistic experiment indicates that if the male and female are 4 m apart on a connected perch, females tremulate, and never perform phonotaxis while males perform vibrotaxis. However, at a distance of 9 m, 4 out of 10 females begin phonotaxis after a period of tremulation. We then hypothesized that features of the male call that indicate caller distance, such as call sound pressure level (SPL), might be responsible for this distance-dependent variation in the choice between phonotaxis and tremulation However, we found that at all SPLs, the female tremulates in response to male calls before attempting phonotaxis and that the probability of phonotaxis and tremulation both increased with calling song SPL. We conclude that our first hypothesis is upheld and that females do behave differently with respect to distance from the male, but that the cue affecting the distance-dependent increase in the probability of initiation of phonotaxis in female response choice is not the SPL of the male's advertisement call.

Keywords: multimodal, duet, acoustic, bushcricket, katydid, Ensifera

INTRODUCTION

Among acoustically communicating Ensiferans, duetting species depart from the standard Ensiferan paradigm of female phonotaxis to male calls. Among duetting Ensiferans as well as in various other lineages of duetting insects, females produce a signal in response to male calls, and male taxis to the female response call is the most common form of localization for acoustically duetting cicadas, visually duetting fireflies, and vibrationally duetting lacewings and stoneflies (Bailey, 2003).

However, other response modes exist and the relative prevalence of male and female phonotaxis varies across and even within acoustically duetting Ensiferan species (Bailey, 2003). In some species such as *Elephantodeta nobilis* (Bailey and Field, 2000), *Amblycorypha parvipennis* (Galliart and Shaw, 1996), *Amblycorypha rotundifolia, Montezumina modesta,* (Spooner, 1995), *Microcentrum rhombifolium, Scudderia texensis,* (Spooner, 1968), *Steropleurus stali* and *Steropleurus nobrei* (Hartley, 1993), *Isophya rossica, Isophya stepposa,* and *Isophya taurica* (Zhantiev and Dubrovin, 1977; Zhantiev and Korsunovskaya, 1986), the sexes can approach each other by performing mutual phonotaxis to each other's calls.

Mutual phonotaxis can occur simultaneously or in a particular order, varying with respect to distance and call sound pressure levels (SPL). For example, in *Microcentrum rhombifolium, Amblycorypha oblongifolia,* and *Scudderia texensis*, females perform phonotaxis only to low SPL calls indicative of distant males (Spooner, 1968).

In many species the male calling songs are complex, with a particular part of the call eliciting a temporally specific female acoustic response (Heller and von Helversen, 1986; Bailey and Hammond, 2003). The male call may also vary over the time course of the duetting and localization process, and with respect to the female's response. In *Amblycorypha floridana* and to some extent *Montezumina modesta,* the male produces two types of sounds, a short lisp that elicits a high SPL acoustic response from the female toward which he partially moves (Spooner, 1995), and then he produces another longer high SPL lisp which elicits a phonotactic response from the female (Spooner, 1968). This can occur in the reverse order in some species such as *Scudderia texensis* and *Microcentrum rhombifolium*, where the female partially approaches the source of the male call, by when he begins the second call type to which she produces (low intensity) acoustic responses, which then attract him to move the rest of the way toward her (Spooner, 1968). In these cases, the male and female both effectively share the burden of localization.

On the other hand, in the duetting phaneropterine *Poecilimon ornatus,* the male moves toward the female while she stays stationary and produces responses to the male call, and males reduce their call SPL as they approach females (Helversen et al., 2001). Likewise, *Scudderia curvicauda* males produce high SPL songs when low SPL answering calls are broadcast to them, and reduce their calling SPL as they do phonotaxis (Spooner, 1968). In these cases, call SPL would not be indicative of male distance.

Males of the ephippigerine species *Steropleurus stali* (Bateman, 2001) and *Platystolus obvius* (Hartley et al., 1974) increase their calling rate once the female engages in a duet, and similarly *Scudderia texensis, S. furcate, and S. cuneata* males increase the number of successive pulses in their songs. There is some indication that some aspects of the acoustic call may be used as a cue for distance. Male *Leptophyes punctatissima* only move toward females (Hartley and Robinson, 1976) whose answering call SPL is over 50 dB SPL (Zimmermann et al., 1989), and whose overall response latency falls within a narrow window of 20–50 ms relative to the male call (Robinson et al., 1986). Both the calling SPL and the overall response latency vary with respect to the distance between the duetting pair, and successful phonotaxis also varies sharply with respect to distance (Zimmermann et al., 1989). The latency of the female responses is remarkably low and reliable for each individual *Leptophyes* female, but the general phenomenon of species-specific latencies of female replies being necessary for male phonotaxis holds for many acoustically duetting Ensiferans (Bailey, 2003). With longer latencies, there is potential for silent satellite males to insert their own brief trigger pulses that can elicit a female acoustic response into the intervals between chirps of the male call, as is seen in *Elephantodeta nobilis* (Bailey and Field, 2000).

The multimodal duetting communication system of the pseudophylline bushcricket *Onomarchus uninotatus* involves male acoustic and female vibratory signals (Rajaraman et al., 2015). The male's acoustic call elicits bouts of tremulation from the females that have a specific temporal relationship of alternation with the acoustic chirps. This female tremulation transmits a vibrational signal along the substrate, which the male can detect and use to localize the female by performing vibrotaxis. The male performs vibrotaxis by tracking the vibrational component of the duet, but does not move toward the same vibrational signal if the acoustic component of the duet is missing (Rajaraman et al., 2015). This pattern of male vibrotaxis to the female vibrational signal is also seen in lebinthine crickets described by Ter Hofstede et al. (2015). However, the female *O. uninotatus* can also perform phonotaxis to the male call, after a period of tremulation (Rajaraman et al., 2015). Two possible forms of localization might therefore operate in *O. uninotatus:* female phonotaxis to the male acoustic call, or male vibrotaxis to the female's tremulation in response to his acoustic call, depending on the female response choice between tremulation and phonotaxis. Since a vibrational signal would not transmit well across trees and *O. uninotatus* is a canopy dweller, we hypothesized that the female response mode might vary with distance from the male. We also predicted that females would preferentially respond with phonotaxis to low SPL male calls (indicative of a distant male) and that tremulation would be the preferred response to high SPL calls. We first investigated the spatiotemporal dynamics of duetting in a semi-natural setup with the duetting pair initially separated by different distances, and then examined female responses to played back calls of varying SPLs.

MATERIALS AND METHODS

Onomarchus uninotatus were caught as nymphs from plantations of *Artocarpus* sp. in Kaddari village, Karnataka, India (latitude 13°13'N, longitude 75°5'E), between December 2011 and May 2012 and then again between November 2016 and May 2018. The nymphs were reared on *Artocarpus heterophyllus* leaves and water in cylindrical plastic boxes (diameter 15 cm, height 17 cm) in the laboratory at the Indian Institute of Science, Bangalore at room temperature (18–24 deg C) and a natural 12 h:12 h light:dark cycle as described in Rajaraman et al. (2015).

Experiments were carried out on virgin males at least 1 week after the final molt and females at least 10–15 days after the final molt, at which point they became responsive to male calling song.

The SPLs reported for the male call were measured in the wild with a handheld SPL meter (Brüel and Kjær Observer 2260, Denmark) with a 1/2 inch microphone (Brüel and Kjær 4189, Denmark, frequency response range 6 Hz—20 kHz), 0.5 m from below and behind the calling male.

Semi-natural Experiment on Duetting

An experiment on the distance dependent dynamics of duetting behavior was conducted in a semi-natural setup, with the female and male separated by a distance of either 4 or 9 m. For the 4 m setup, 7 of the 11 trials were conducted in a 4 m*3 m*3.5 m cage that was built outdoors around a 4 m long live *Artocarpus heterophyllus* branch. For the 9 m treatment, a 10 m*3 m*4 m cage was built outdoors around a cut *Artocarpus heterophyllus* branch of length 9.2 m. The branch was stripped of side-branches, and the branch split into two about 1 m from its tip. The same 9.2 m long cut branch was used in a 4 m treatment for 4 trials. After checking that there were no significant differences in the latency of female tremulation, male vibrotaxis and localization between the cut and the intact branches at 4 m across the cut and live branch trials (**Supplementary Figures 1–3**), these data were pooled.

The experiment was conducted in the night between 1930 and 0030 h between January 2017 and May 2018. The males and females were kept separated in the experimental arenas at least 2 h before each experimental trial in order to acclimatize them to the conditions in the arena. For each trial the males were released beforehand on the branch and the females were released at the appropriate distance after the male started calling. The sequence of events was recorded with male and female behavior separately monitored using a Canon XA-10 Professional Camcorder and Sony Handycam HDR-XR 500 in the night shot mode. The end of the trial was marked by the mutual co-localization of the pair. Only one trial was done per night and animals were not repeated across nights.

The behavior of the duetting couple was then coded by the combination of behavioral states exhibited by males and females. The combined behavioral state of the pair was assessed by analyzing the videos of each individual of the communicating pair, and marking the behavioral state demonstrated by the duetting pair during each 1 s bin. The transition probability between behavioral states was then calculated using a first order Markov model in R (Markovchain package, v. 0.6.9.10), which assumes that every state is determined only by the state preceding it, and given an input of a series of states, calculates the transition probability between behavioral states.

In order to assess the normality of data in the two groups (4 and 9 m), a Shapiro-Wilk test was performed and the data in the two groups were found to be non-normally distributed. Comparisons between responses at different distances were checked with a Wilcoxon rank sum test.

Female Responses to Varied Call SPL

An experiment investigating the effect of changing song SPL on female response choice was carried out in an anechoic chamber, on whose floor lay a T shaped structure consisting of a 1 m jackfruit branch of relatively uniform diameter nailed to the center of another 2 m long branch, with all 3 ends placed on blocks of black acoustic foam. In each trial, a randomly chosen female was placed on the end of the jackfruit branch leading to the T junction, such that either turn would lead to a 1 m long walk toward an X-mini speaker (v1.1, XMI Pvt. Ltd, Singapore, frequency range 120 Hz to 20 KHz) placed at either end of the 2 m long branch. At any given trial only one randomly selected speaker would be used to play the male call, as described in Rajaraman et al. (2015). The experiment was conducted between 2100 and 0300 h from February to May.

Females were subjected to a randomly ordered series of 8 trials, each with a different stimulus: the conspecific natural pre-recorded *Onomarchus uninotatus* call played back at one of the following sound pressure levels (SPL): 36, 40, 46, 56, 66, or 76 dB SPL (re. 2^*10^{-5} N/m^2); a silent control, or a heterospecific call control (*Gryllacropsis* call, frequency 1.7 kHz) played back at 66 dB SPL. *Onomarchus uninotatus* and *Gryllacropsis* sp. calls were sourced from those made by Diwakar and Balakrishnan (2007). The single recorded male call played back to all females in the SPL experiment (**Supplementary Figure 4**) was the same as that used in Rajaraman et al. (2015) and had a mean calling period of 1.203 ± 0.01 s (mean ± s.d., $n = 6$ chirps played on repeat), while the calling period typical of all male calls described by Diwakar and Balakrishnan (2007) was 1.17 ± 0.15 s (mean ± s.e., averaged across $n = 6$ animals). The number of syllables per chirp in our call was 3, which was typical of animals we heard calling in the wild, and recordings made in the wild by Diwakar and Balakrishnan (2007) indicate a mean of 2.32 ± 0.47 syllables per chirp (mean ± s.e., averaged across $n = 6$ animals); accordingly the average chirp duration they report is 0.15 ± 0.04 s. This is similar to the duration of 2 syllables of the chirp in the male call we played back; but with the third syllable included, the average duration of our call was 0.210 ± 0.001 s (mean ± s.d., $n = 7$ chirps). The syllable duration in the male call we played was 0.044 ± 0.001 s (mean ± s.d., $n = 21$ syllables), comparable to the duration of 0.044 ± 0.004 s (mean ± s.e., $n = 6$ animals) measured by Diwakar and Balakrishnan (2007). The peak calling frequency of our played back call was 3.36 kHz, and the average dominant calling frequency reported by Diwakar and Balakrishnan (2007) was 3.23 ±0. 0.1 kHz, with a bandwidth of 0.4 ± 0.06 kHz (mean ± s.e., $n = 6$ animals). The SPL reported in the lab experiment was measured at the female's initial location by a handheld sound level meter (Brüel and Kjær Observer 2260, Denmark) with a 1/2 inch microphone (Brüel and Kjær 4189, Denmark, frequency response range 6 Hz to 20 kHz). The call was relayed to the speaker from a laptop (Acer Aspire S3, Acer, Taiwan), and played out through Audacity free software (v. 2.1.2, GNU GPL) at a sampling rate of 44.1 kHz. The response of the animal to each stimulus was recorded for 5 min with a videocamera (Sony Handycam DCR-HC96E, Japan) in the night shot mode. Videos were digitized using Microsoft Windows Movie Maker software (v. 5.1, Microsoft Corporation, USA) onto a HP laptop (Compaq nx6320, Hewlett-Packard, USA) on which the videos remain available for reference. Consecutive trials were separated by a minimum of 5 minutes. The female was classified as having performed phonotaxis if she walked all the way to a speaker.

Differences between the numbers of animals responding to different treatments were tested with a McNemar's test, applying the Yates correction, the null hypothesis being that the females responded equally to all stimulus types. The latency of onset and the duration of tremulation and phonotaxis were noted for each treatment and comparisons were made using Welch's paired t-tests after the normal distribution of differences between treatments was checked using a Shapiro-Wilk test. To compare the number of tremulations at 40 dB SPL vs. the silent control, since the differences were not normally distributed, a Wilcoxon paired signed-rank test was performed.

RESULTS

Female Response Choice Varies With Distance From Duetting Male Partner in Semi-natural Conditions

The results of the semi-natural experiment are depicted in an ethogram (**Figure 1**). The behavioral state at the beginning of the experiment always involved the female being released onto a branch, at a set distance away from the already calling male. The female would sit for a brief period of time without tremulation while the male sang, and soon after this female tremulation began. This multimodal duet was occasionally interrupted, with either pauses in the female's tremulatory response, or pauses in male call. When the male call stopped, females would sometimes stop tremulating; sometimes the female would continue to tremulate in silence (states A-C, **Figures 1A,B**). As with the initial experiments on female *O. uninotatus* (Rajaraman et al., 2015), tremulation was always her first response to the male call.

At a separation of 4 m, the state of male calling and female tremulation transitioned into a state where the male began vibrotaxis ($n = 11$) (state D, **Figure 1A**). In some cases, the male began vibrotaxis while continuing to call, but the call structure changed to one with a shorter number of syllables, usually just 1 syllable (**Supplementary Figure 5**), eventually always leading to the state of silent vibrotaxis by the male (state G, **Figure 1A**). The female continued to stay stationary during male vibrotaxis, sometimes tremulating when the male called, and sometimes tremulating even in the absence of the acoustic call. While the female occasionally walked around, it was never in response to the male call, and so at 4 m, females never performed phonotaxis.

The females' first response is tremulation in both the live and cut branch setups at 4 m, and no phonotaxis seen in both. Mate-search is solely performed by males in both setups. No statistically significant differences were seen in the latency of female tremulation (**Supplementary Figure 1**), male vibrotaxis (**Supplementary Figure 2**) or the latency of localization by males in the cut vs. live branch (**Supplementary Figure 3**), although there is a lot more variation seen in the localisation latency in the cut branch.

At a separation of 9 m, however, 4 out of 10 females performed phonotaxis. The initial part of the behavioral sequence is similar to that at 4 m, with the male calling, and the female commencing tremulation, with occasional interruptions in the duet (states A–C, **Figure 1B**). Males sometimes performed vibrotaxis as happened with the 4 m treatment of the semi-natural experiment, sometimes calling on the way, usually with an abbreviated call structure (states D, G, **Figure 1B**; **Supplementary Figure 5**). However, in 2 cases the females ended their tremulation and began phonotaxis while the male called, before the male commenced moving (state F, **Figure 1B**). In the 2 other cases, the female began phonotaxis while the male was performing vibrotaxis and, while walking, he produced the changed call (**Supplementary Figure 5**). In all cases where the female performed phonotaxis at 9 m, she did not walk all the way toward the male, and instead paused and tremulated in response to his call if it arrived when she was stationary.

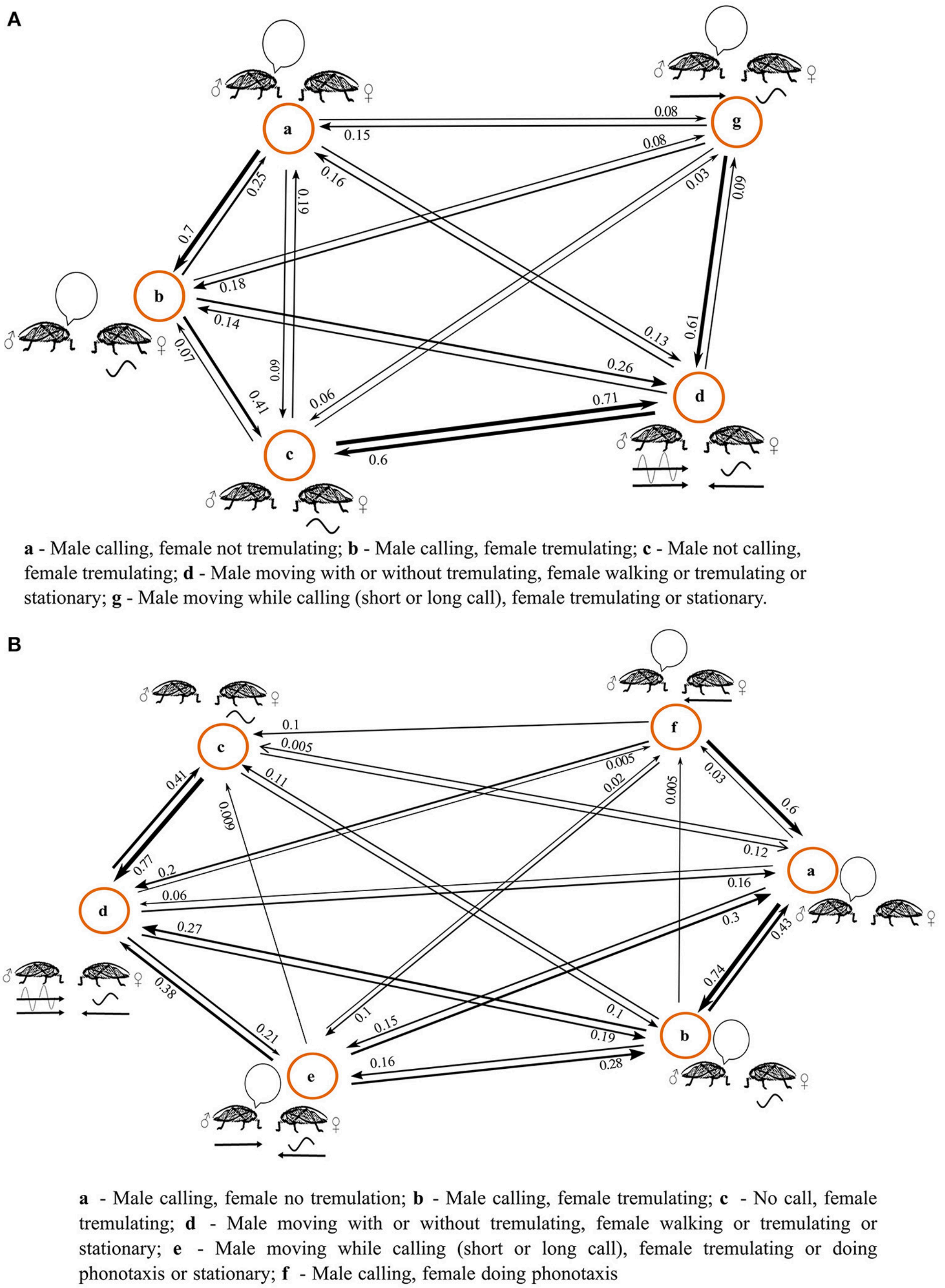
At both 4 and 9 m, the transition probability matrix of observed behavior was found to be non random and significantly different from the expected transition probability matrix if the sequence of behavior were to be random and all transitions were equally likely, as tested using a chi square goodness of fit-test (at 4 m, $\chi 2 = 503.1$, df $= 16$; at 9 m $\chi 2 = 780.3$, df $= 25$).

Female Response Mode Does Not Change With Acoustic Call SPL

Since females showed varied responses to the male call depending on their distance from the male, female responses were tested to male acoustic calls played back at a range of sound pressure levels (SPLs). A robust tremulation response to the conspecific call was seen within the first minute of onset of the male call for all individuals at 66 dB SPL. The number of animals that respond to the conspecific call within the first minute is lower at other tested SPLs, reducing monotonically with reducing stimulus SPL. The relationship between the proportion of females out of the total number of 16 who tremulate within the first minute of the onset of the male acoustic call, and SPL, can be fit by a saturating inverse exponential relationship (**Figure 2A**).

Females never tremulated in silence prior to being exposed to the male call. The number of females that tremulate to the 40 dB SPL playback of the conspecific call is significant compared to the number responding to silence ($\chi^2 = 8.1$, df $= 1$, $p = 0.0044$). This is also true for the number of females tremulating at all higher SPLs in comparison to the number tremulating in silence (in all cases $p < 0.001$). However, the number of females that tremulate at 35 dB SPL is not significantly different from the number tremulating in silence, suggesting that the threshold of the response lies between 35 and 40 dB SPL.

The median number of female tremulatory responses observed during the first minute following the onset of playback of the conspecific male acoustic call rises monotonically with conspecific stimulus SPL upto 66 dB SPL. At 76 dB SPL, the highest tested SPL, we see a decrease in the number of tremulation events, although this decrease is not significant (**Figure 2B**).



a - Male calling, female not tremulating; **b** - Male calling, female tremulating; **c** - Male not calling, female tremulating; **d** - Male moving with or without tremulating, female walking or tremulating or stationary; **g** - Male moving while calling (short or long call), female tremulating or stationary.

a - Male calling, female no tremulation; **b** - Male calling, female tremulating; **c** - No call, female tremulating; **d** - Male moving with or without tremulating, female walking or tremulating or stationary; **e** - Male moving while calling (short or long call), female tremulating or doing phonotaxis or stationary; **f** - Male calling, female doing phonotaxis

FIGURE 1 | Ethograms of the behavior of the duetting pair at initial separation distances of **(A)** 4 m ($n = 11$) and **(B)** 9 m ($n = 10$), constructed using a First Order Markov Model. The numbers next to the arrows are probability values which give (NOT gives) the probability of transitioning from one behavior to the other. The thicknesses of the arrows are in accordance with the probability values they represent.

The number of tremulatory responses at 40 dB SPL is significant relative to the response to silence (Wilcoxon signed-rank $V = 50$, $p = 0.01$), as are the responses for 46 dB SPL and above (in all cases $p < 0.001$), while the response at 35 dB SPL is not significantly different from the response to silence. This corroborates the estimate of a tremulation threshold lying between 35 and 40 dB SPL.

The proportion of females out of a total of 16 that show a phonotactic response to the conspecific call is lower at each SPL than the proportion of animals that show a tremulation response. While the proportion of animals responding with phonotaxis to the conspecific call does not rise monotonically with call SPL even up to 66 dB SPL, the relationship can be fit by a saturating inverse exponential (**Figure 3**). The number of animals showing

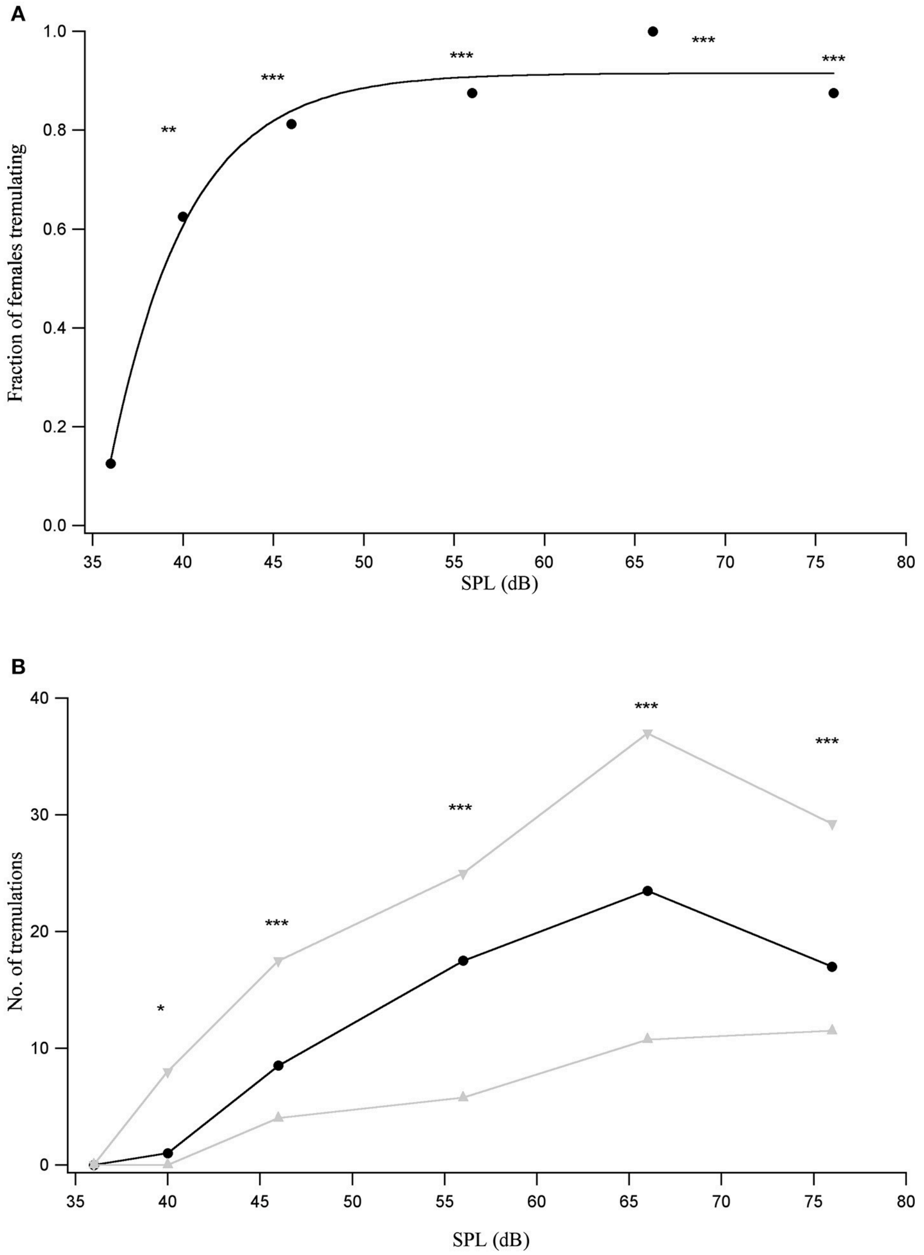


FIGURE 2 | (A) Proportion of animals that tremulate in response to the conspecific call played back at different SPLs ($n = 16$). $^{**}p < 0.01$, $^{***}p < 0.001$ for comparisons of response at a given SPL with the response to silent controls. **(B)** Median number of tremulation events (black trace) observed across animals in the first minute following the onset of the conspecific male acoustic call, played back at different SPLs, with the interquartile range indicated in gray. $^{*}p < 0.05$, $^{***}p < 0.001$ for pairwise comparisons with the response to a silent control.

a phonotactic response to the conspecific call is only significantly higher than the number walking during a silent control, when the call is played back at 66 dB SPL ($\chi^2 = 8.1$, df $= 1$, $p = 0.0044$) and 76 dB SPL ($\chi^2 = 8.1$, df $= 1$, $p = 0.0044$).

The calling SPL of the males is fairly similar across individuals, measured to be about around 71.9 ± 0.5 dB SPL ($n = 5$) measured 50 cm from behind the animal in the field. We interpolate the call SPL that the females would hear in the semi-natural experiment using an attenuation of 6 dB SPL per doubling of distance, to find that at a 4 m horizontal distance the female would hear the male acoustic call at 54 dB SPL, while at 9 m she would hear the call at 47 dB SPL. The proportion of females doing phonotaxis at 46 dB SPL (lab experiment) and at 47 db SPL (9 m treatment, semi-natural experiment) are similar. Interestingly, however, the proportion of females doing phonotaxis at a distance of 4 m and 54 dB SPL in the semi-natural experiment is zero, unlike the proportion of females who perform phonotaxis in the lab during playback of the acoustic call at 56 dB SPL (**Figure 3**). This suggests that SPL of the advertising call is not the cue mediating distance dependent variations in female response choice in the semi-natural experiment.

We examined the latency of the onset of both tremulatory (**Figure 4A**) and phonotactic (**Figure 4B**) responses after song onset at different SPLs of conspecific call playback, to see whether male acoustic call SPL affected the duration for which females tremulated before beginning phonotaxis. We found no significant differences across SPLs above threshold. However at all SPLs, the latency of the first tremulation event was an order of magnitude lower than the latency of phonotaxis (**Figure 4**).

Since tremulation always preceded phonotaxis, we proceeded to investigate whether vibrotaxis similarly was likely to precede phonotaxis. The time taken for a calling male to initiate vibrotaxis was assessed in the semi-natural experiment, since vibrotaxis was initiated in response to female tremulation, whereas the time taken to initiate phonotaxis was assessed in the laboratory experiment where calls were played back continuously. All vibrotaxis latencies are calculated from the onset of female tremulation, while phonotaxis latencies are calculated from the

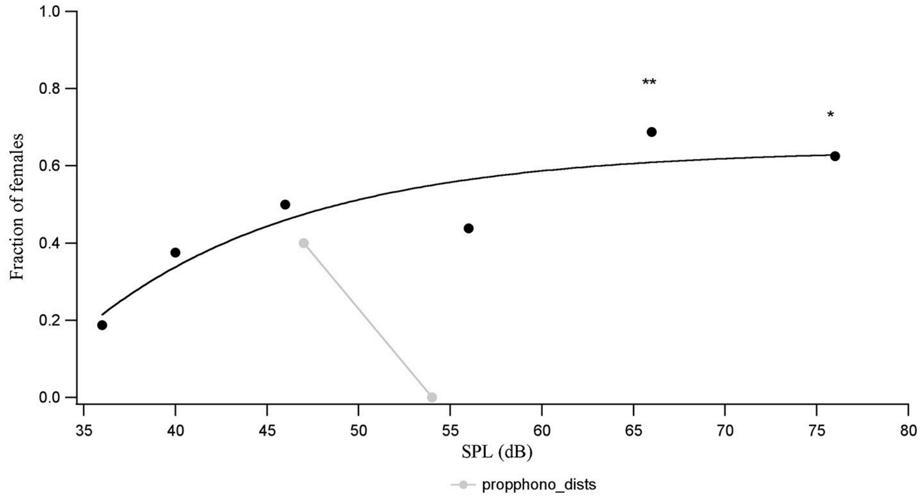


FIGURE 3 | The proportion of animals ($n = 16$) who complete phonotaxis vs. conspecific call SPL, compared to the proportion of animals seen to complete phonotaxis in the semi-natural experiments at 4 and 9 m distances (corresponding to acoustic call SPLs of 54 and 47 dB SPL, respectively). $^{*}p < 0.05$ and $^{**}p < 0.01$ for comparisons of response at a given SPL with the response to silent controls.

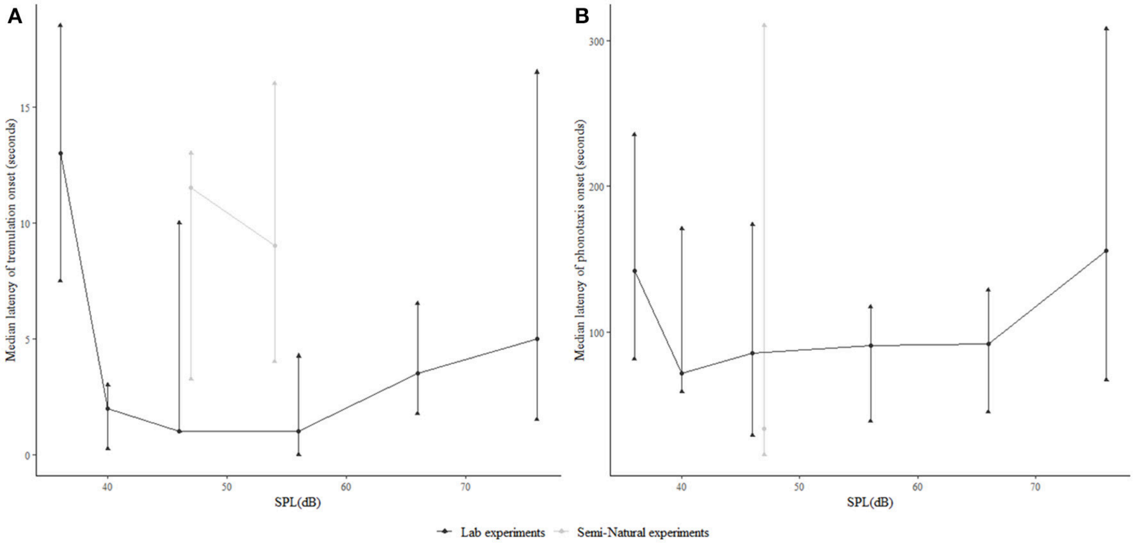


FIGURE 4 | Relationship of the latency of **(A)** onset of female tremulation and **(B)** the onset of female phonotaxis after song onset, in the laboratory as well as semi-natural settings, relative to stimulus SPL.

onset of the male acoustic call. At 54 dB SPL, corresponding to a 4 m distance, the time taken to initiate vibrotaxis in the semi-natural experiment was significantly shorter than the time taken to initiate phonotaxis at 56 dB SPL in lab experiments, whereas at 9 m, vibrotaxis at 47 dB SPL did not commence significantly more quickly than phonotaxis as assessed in the laboratory playback experiment at 46 dB SPL. When the semi-natural experimental trials where the female began phonotaxis were separated from trials where she did not do phonotaxis (in order to check that at all SPLs, comparisons of latency were made under conditions where the origin of the signals was stationary), there was still no significant difference in the latency of onset of male vibrotaxis and female phonotaxis at 9 m (**Figure 5**).

Vibrotaxis is the only form of localization seen in the semi-natural experiment at a separation of 4 m, while it is the dominant form of localization at a separation of 9 m. The time taken for the completion of vibrotaxis by the male and localization of the female in the semi-natural experiment, however, is significantly higher than the latency of onset of female phonotaxis in the laboratory experiment, at both distances and SPLs (**Figure 6**). This suggests that at 4 m, while vibrotaxis typically begins more swiftly than phonotaxis to sound played back at an equivalent SPL, it is not completed quickly enough to explain the failure of the female to begin phonotaxis at that SPL. When vibrotaxis and phonotaxis both happen, colocalization is significantly quicker ($p = 0.02$) than when only one form of localization takes place.

DISCUSSION

Female Response Choice Varies With Distance From Duetting Male Partner

The results of the semi-natural experiment support our hypothesis that the mode of female response and the sequence of behaviors involved in mutual localization are affected by the initial distance separating the duetting pair. In a choice between responding to the male acoustic call with tremulation and phonotaxis, it is clear that tremulation is the more likely and more immediate female behavioral response, in both the semi-natural and laboratory conditions. Only a subset of the females who tremulate perform phonotaxis. In the semi-natural condition phonotaxis occurs only at a separation of 9 m. At a separation of 4 m, however, not a single female does phonotaxis. This clearly indicates that the female employs different strategies and modes of response at different initial separation distances from the male.

Female Response Mode Does Not Vary With the SPL of the Male's Acoustic Call of Advertisement

Our second hypothesis looked at SPL. In investigating how the choice between phonotaxis and tremulation varied with stimulus SPL, we were testing the hypothesis that louder calls would preferentially elicit a tremulation response and no phonotaxis, of the kind seen at 4 m distances, while lower SPL calls would more likely elicit a phonotactic response.

We do not find support for the hypothesis that SPL affected the choice between tremulatory and phonotactic behavior because the timing of onset of both tremulation and phonotaxis did not vary significantly with SPL. Phonotaxis always followed a period of tremulation and the latency of onset did not decrease with decreasing SPL. The time taken to complete phonotaxis did not vary significantly with SPL either.

The probability of phonotaxis and the probability of tremulation both increased with SPL up to 66 dB SPL. Therefore, at high SPL calls indicating proximal male callers, a female is more likely to tremulate, and more likely to do phonotaxis. When she tremulates, the number of tremulations she performs within the first minute of the onset of the male acoustic call goes up with increasing call SPL. This is not surprising at a neurophysiological level, because all responses could be expected to increase with sensory stimulus levels. Phonotaxis in particular has been shown to generally improve with increasing stimulus levels (Ulagaraj and Walker, 1975; Walker and Forrest, 1989; Forrest and Green, 1991). But at all SPLs above threshold, the probability of female tremulation was higher than the probability of phonotaxis, suggesting that these two behaviors do not trade-off in terms of probability along a range of advertisement male acoustic calling song SPLs.

Response Threshold and Overload

An exception to the increase in probability and frequency of tremulation, and the probability of phonotaxis with SPL, is the decrease in these parameters going from 66 dB SPL to 76 dB (**Figures 2**, **3**). A call played back at 76 dB SPL may therefore represent an example of unnatural sensory overload. Ulagaraj and Walker (1975), however, found that calls as much as 6 dB louder than the natural calling SPL of 100 dB attracted more mole cricket females of the species Neo*scapteriscus borellii* in sound traps than the called played at the natural SPL of 100 dB. Beyond 106 dB, the number of females attracted to the sound trap plateaued. Sensory overload may unsurprisingly differ in its attractive or aversive value between various Orthopterans.

The threshold of the tremulation response was estimated from the SPL at which both the frequency and probability of tremulation in response to the conspecific call differ significantly from behavior under the silent control condition (**Figure 2**). For both parameters the threshold estimated this way lay between 35 dB SPL where the response was not significantly different from the response under silence, and 40 dB SPL where the response was significantly different.

Phonotactic Suppression at Short Distances in Semi-natural Conditions

In the semi-natural experiment, as opposed to the laboratory experiment, phonotaxis is not in fact more likely as call SPL increases. Phonotaxis was never observed in the semi-natural experiment at a separation of 4 m, but was observed at a separation of 9 m. In the laboratory experiments where we manipulate acoustic call SPL, we do not see a reduced probability of phonotaxis for high-SPL calls that might indicate a nearby male. This is in contradiction with the reduced probability of phonotaxis seen in the semi-natural experiment at a shorter

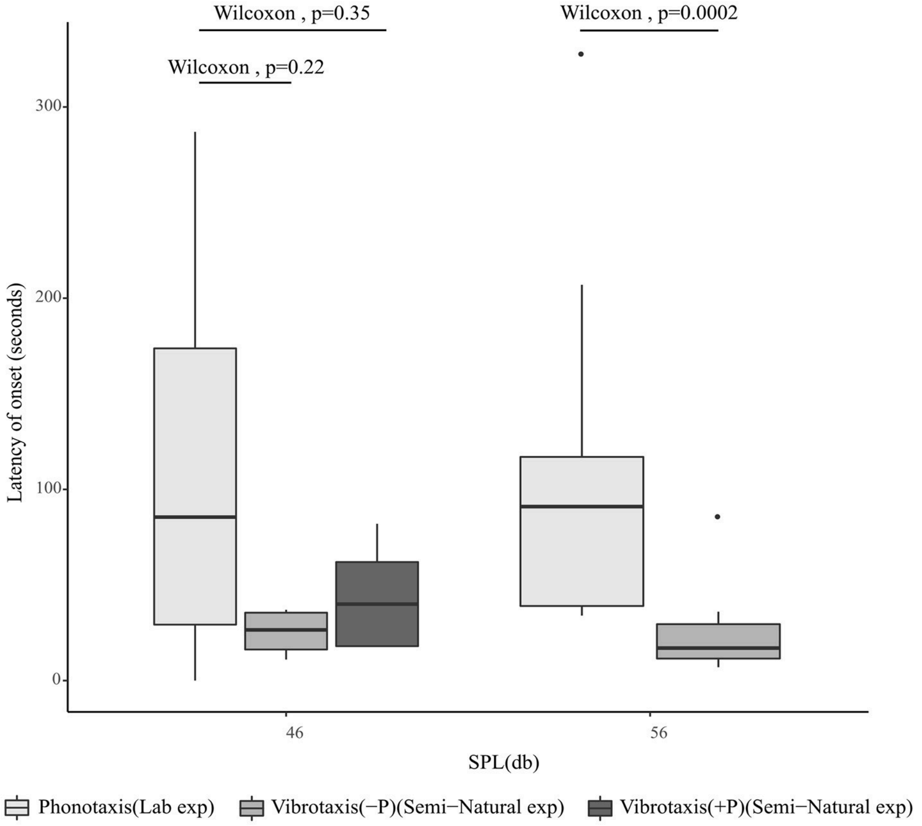


FIGURE 5 | Median latency of onset of female phonotaxis assessed in the laboratory experiment at 46 and 56 dB SPL where the position of the sound emitting speaker does not move ($n = 13$), compared to time taken for the onset of male vibrotaxis as assessed in the semi-natural experiment at 4 m (54 dB SPL) ($n = 11$) and 9 m (47) dB SPL ($n = 10$). For 4 m, the source of the vibrational signal was always stationary; for 9 m, vibrotaxis data are segregated by whether the female did (+P) ($n = 4$) or did not perform phonotaxis (–P) ($n = 6$). All vibrotaxis latencies for this plot are calculated from the onset of female tremulation, while phonotaxis latencies are calculated from the onset of the male acoustic call.

distance and high call SPL. The difference between these results and those showing increasing phonotaxis probability with calling SPL in the laboratory experiment suggests that the SPL of the advertisement call is not the cue mediating the difference in female response modes at different distances.

Specifically, the similarity in the probability of phonotaxis seen in natural and laboratory experimental results at 46–47 dB SPL (**Figure 3**), and the divergence at higher SPLs suggests that at higher SPLs, other cues indicating the proximity of the pair might reduce the probability of female phonotaxis. One such cue might be the cessation of the male call in response to female tremulation, prior to beginning vibrotaxis. While this cue differs between the laboratory and semi-natural experiments, we would expect such a cue to suppress phonotaxis both at 4 and 9 m separations. Likewise, outdoor conditions of background noise, temperature and humidity differ between the semi-natural and laboratory experiments, but would not be expected to vary between the 4 and 9 m treatments of the semi-natural experiment.

A simple possibility is that the female does not perform phonotaxis in the 4 m treatment of the semi-natural experiment because the male reaches her before she begins to move. But in two cases out of four examples of phonotaxis at a 9 m separation in the semi-natural experiment, the onset of female phonotaxis was well after the onset of male vibrotaxis. The other two examples show the onset of female phonotaxis prior to the onset of male vibrotaxis, suggesting that phonotaxis is not simply a delayed response relative to vibrotaxis, commencing only if the male fails to arrive. Furthermore, the average time taken by the male to locate the female 4 m away is significantly longer than the time it would have taken her to begin phonotaxis in the lab experiment where we played back the male calling song (**Figure 5**). The absence of phonotaxis at 4 m can therefore not be explained by the female waiting for the male to actually find her. This adds weight to the possibility that phonotaxis in semi-natural conditions is suppressed by cues at short distances, rather than the possibility that females simply do not commence phonotaxis at short distances because the male arrives before she would.

In all cases in the 4 m treatment of the semi-natural experiment, male vibrotaxis was likely to commence earlier than female phonotaxis latency as measured in the lab playback experiment (**Figure 6**). Therefore, other cues from the walking

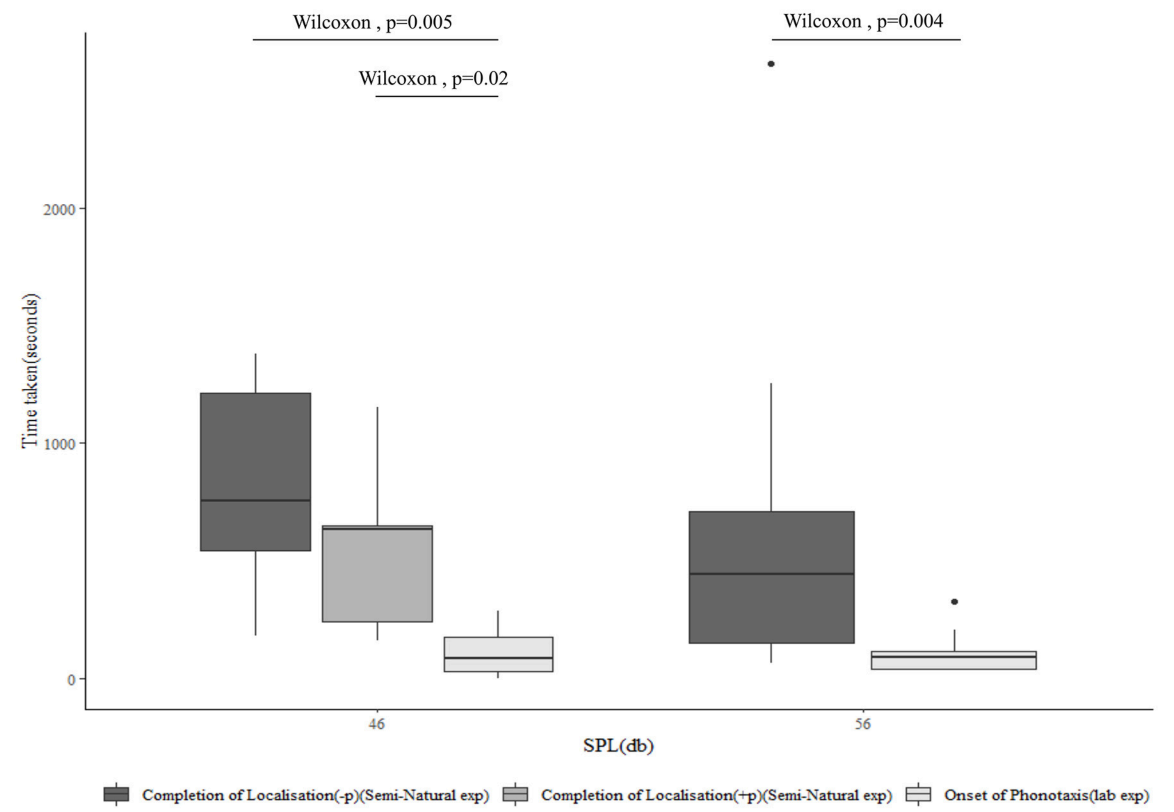


FIGURE 6 | The median latency of onset of phonotaxis as assessed in the laboratory experiment at 46 and 56 dB SPL (*n* = 13), compared to time taken for the completion of vibrotaxis and localization of the female by the male as separately assessed in those treatments of the semi-natural experiment where the female does not perform phonotaxis, at 4 m (54 dB SPL) (*n* = 11) and 9 m (47) dB SPL (*n* = 10), and where the female does perform phonotaxis at 9 m (*n* = 4). All vibrotaxis latencies for this plot are calculated from the onset of female tremulation, while phonotaxis latencies are calculated from the onset of the male acoustic call.

male might exert a distance dependent effect. Vibrational cues from the male, or the changes in the male acoustic calling structure while he walks, might provide cues to the female that suppress the probability of her commencing phonotaxis. All these cues are present only in the semi-natural experiment and not in the lab experiment. Since both of these decay with transmission distance, they might be of lower intensity at 9 m and exert a suppressive effect only at 4 m.

Another possibility is that olfactory or visual cues mediate male taxis toward the female at short distances. Our occasional observations of males walking toward females even before commencing a broadcasting call at short distances (1–4 m) suggests that non-auditory cues might play a role. The semi-natural experiment was conducted outdoors and so light was not completely controlled as a factor and neither was smell. However, in the laboratory playback experiment, both light and smell were controlled for. Dim light is known to affect phonotaxis in Ensiferans (Bohm et al., 1991; von Helversen and Wendler, 2000), as well as courtship song in grasshoppers (Riede, 1986).

As the male walks toward the female, the call structure changes, but it is also possible that call SPL varies—it could become louder as the male moves closer, or softer. While we have video recordings of the experiment that include an audio component, these data cannot provide a proper calibrated quantification of the change in SPL as the male moves. Our study was also limited by the relatively difficult comparison between the complexity of signaling in semi-natural settings, which is clear from the ethograms (**Figure 1**) and the laboratory experiments involving the manipulation of acoustic call SPL. Controlling for non-acoustic cues in the semi-natural experiment also proved to be difficult, whereas these could be controlled in the laboratory. Any vibrational non-acoustic cues from the male's walking fell below the threshold of measurement of a Polytec Laser Doppler Vibrometer Polytec Scanning Vibrometer data acquisition unit (VIB-E-220, Polytec GmbH, Waldbronn, Germany) controlled by the vibrometer software (Vibsoft version 4.8, Polytec GmbH, Waldbronn, Germany), and therefore, these could not be quantified. It must be noted that this vibrometer also fails to detect tremulations of an amplitude at which the males respond, and so the detection threshold of the males is lower and more sensitive than this vibrometer. The question of whether non-acoustic cues mediate phonotactic suppression, and if so, which cues could be investigated more precisely in further studies with more sensitive equipment that manipulate the presence or absence of each of these cues.

We conclude that female *O. uninotatus* show different modes of response to duetting male partners at different distances, but that acoustic call SPL is not the cue mediating this difference.

DATA AVAILABILITY STATEMENT

All reported behavior has been videorecorded and a copy of these raw data in the form of videos as well as analysis is with the authors, as well as stored at the Center for Ecological Sciences, Indian Institute of Science. The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any researcher.

ETHICS STATEMENT

This study was carried out in accordance with the regulations of the Government of India, and under the guidance of the Institutional Animal Ethics Committee of the Indian Institute of Science. The protocol was approved by the Institutional Research Ethics Committee of the Indian Institute of Science.

AUTHOR CONTRIBUTIONS

RB, KR, and AN designed the experiments, analyzed the results, and wrote the paper together. KR performed the laboratory experiment. AN and AD performed the semi-natural experiment. All authors gave final approval for publication.

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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.2018.00172/full#supplementary-material

Supplementary Figure 1 | The latency of female tremulation at a separation of 4 m from the male in the semi-natural experiment, using the cut vs. live branch.

Supplementary Figure 2 | The latency of male vibrotaxis, at a separation of 4 m from the female in the semi-natural experiment, with the cut vs. live branch.

Supplementary Figure 3 | The time taken for localization by the male of the female, from an initial separation of 4 m in the semi-natural experiment, on the cut vs. live branch.

Supplementary Figure 4 | The sample recorded male call which was played back on repeat to females in the playback experiment.

Supplementary Figure 5 | A sample spectrogram of the change in the male call over the course of a minute, with respect to the onset of tremulation, vibrotaxis, and phonotaxis, in the 9 m treatment of the semi-natural experiment.

REFERENCES

Bailey, W. J. (2003). Insect duets: their mechanisms and underlying evolution. *Physiol. Entomol.* 28, 157–174. doi: 10.1046/j.1365-3032.2003.00337.x

Bailey, W. J., and Field, G. (2000). Acoustic satellite behaviour in the Australian bushcricket *Elephantodeta nobilis* (*Phaneropterinae*, *Tettigoniidae*, *Orthoptera*). *Animal Behav.* 59, 361–369. doi: 10.1006/anbe.1999.1325

Bailey, W. J., and Hammond, T. J. (2003). Duetting in insects – does call length influence reply latency? *J. Zool.* 260, 267–274. doi: 10.1017/S0952836903003728

Bateman, P. W. (2001). Changes in phonotactic behavior of a bushcricket with mating history. *J. Ins. Behav.* 14, 333–343. doi: 10.1023/A:1011167128430

Bohm, H., Schildberger, K., and Huber, F. (1991). Visual and acoustic course controling the cricket *Gryllus bimaculatus*. *J. Exp. Biol.* 159, 235–248.

Diwakar, S., and Balakrishnan, R. (2007). The assemblage of acoustically communicating crickets of a tropical evergreen forest in Southern India: call diversity and diel calling patterns. *Bioacoustics* 16, 113–135. doi: 10.1080/09524622.2007.9753571

Forrest, T. G., and Green, D. M. (1991). Sexual selection and female choice in mole crickets (*Scapteriscus: Gryllotalpidae*): modelling the effects of intensity and male spacing. *Bioacoustics* 3, 93–109. doi: 10.1080/09524622.1991.9753166

Galliart, P. L., and Shaw, K. C. (1996). The effect of variation on parameters of the male calling song of the katydid, *Amblycorypha parvipennis* (*Orthoptera*: *Tettigoniidae*), on female phonotaxis and phonoresponse. *J. Insect Behav.* 9, 841–855. doi: 10.1007/BF02208973

Hartley, J. C. (1993). Acoustic behaviour and phonotaxis in the duetting ephippigerines, *Steropleurus nobrei* and *Steropleurus stali* (*Tettigoniidae*). *Zool. J. Linn. Soc.* 107, 155–167. doi: 10.1111/j.1096-3642.1993.tb00219.x

Hartley, J. C., and Robinson, D. J. (1976). Acoustic behavior of both sexes of the speckled bushcricket *Leptophyes punctatissima*. *Physiol. Entomol.* 1, 21–25. doi: 10.1111/j.1365-3032.1976.tb00882.x

Hartley, J. C., Robinson, D. J., and Warne, A. C. (1974). Female response song in the ephippigerines *Steropleurus stali* and *Platystolus obvius* (*Orthoptera*: *Tettigoniidae*). *An. Behav.* 22, 382–389. doi: 10.1016/S0003-3472(74)80035-7

Heller, K. G., and von Helversen, D. (1986). Acoustic communication in phaneropterine bushcrickets: species-specific delay of female stridulatory response and matching male sensory time window. *Behav. Ecol. Sociobiol.* 18, 189–198. doi: 10.1007/BF00290822

Helversen, D. V., Schul, J., and Kleindienst, H. U. (2001). Male recognition mechanism for female responses implies a dilemma for their localisation in a phaneropterine bushcricket. *J. Comp. Physiol. A* 186, 1153–1158. doi: 10.1007/s003590000167

Rajaraman, K., Godthi, V., Pratap, R., and Balakrishnan, R. (2015). A novel acoustic-vibratory multimodal duet. *J. Exp. Biol.* 218, 3042–3050. doi: 10.1242/jeb.122911

Riede, K. (1986). Modification of the courtship song by visual stimuli in the grasshopper *Gomphocerus rufus* (Acrididae). *Physiol. Entomol.* 11, 61–74. doi: 10.1111/j.1365-3032.1986.tb00391.x

Robinson, D., Rheinlaender, J., and Hartley, J. C. (1986). Temporal parameters of male–female sound communication in *Leptophyes punctatissima*. *Physiol. Entomol*. 11, 317–323. doi: 10.1111/j.1365-3032.1986.tb00419.x

Spooner, J. D. (1968). Pair-forming acoustic systems of phaneropterine katydids (*Orthoptera, Tettigoniidae*). *Animal Behav*. 16, 197–212. doi: 10.1016/0003-3472(68)90001-8

Spooner, J. D. (1995). Pair-forming phonotaxic strategies of phaneropterine katydids (*Tettigoniidae: Phaneropterinae*). *J. Orthopteran Res*. 4, 127–129. doi: 10.2307/3503467

Ter Hofstede, H. M., Schöneich, S., Robillard, T., and Hedwig, B. (2015). Evolution of a communication system by sensory exploitation of startle behavior. *Curr. Biol*. 25, 1–8. doi: 10.1016/j.cub.2015.10.064

Ulagaraj, S. M., and Walker, T. J. (1975). Response of flying mole crickets to three parameters of synthetic songs broadcast outdoors. *Nature* 253, 530–531. doi: 10.1038/253530a0

von Helversen, D., and Wendler, G. (2000). Coupling of visual to auditory cues during phonotactic approach in the phaneropterine bushcricket *Poecilimon affinis*. *J. Comp. Physiol. A* 186, 729–736. doi: 10.1007/s003590000126

Walker, T. J., and Forrest, T. G. (1989). Mole cricket phonotaxis: effects of intensity to synthetic calling song (*Orthoptera: Gryllotalpida*: *Scapteriscus acletus*). *Florida Entomol*. 72, 655–659. doi: 10.2307/3495043

Zhantiev, R. D., and Dubrovin, N. N. (1977). Sound communication in the genus *Isophya* (*Orthoptera, Tettigoniidae*). *Zool. Zh*. 56, 38–51.

Zhantiev, R. D., and Korsunovskaya, O. S. (1986). Sound communication in bush crickets (*Tettigoniidae, Phaneropterinae*) of the European Part of the USSR. *Zool. Zh*. 65, 1151–1163.

Zimmermann, U., Rheinlaender, J., and Robinson, D. (1989). Cues for male phonotaxis in the duetting bushcricket *Leptophyes punctatissima*. *J. Comp. Physiol. A* 164, 621–628. doi: 10.1007/BF00614504

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Loser in Fight but Winner in Love: How Does Inter-Male Competition Determine the Pattern and Outcome of Courtship in Cricket *Gryllus bimaculatus*?

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Animal females are generally assumed to prefer males that win fights. However, a growing number of studies in numerous animal taxa demonstrate no correlation between male fighting ability and their attractiveness, or even female preferences for fight losers. One of the methods to measure female preferences employs no-choice tests that evaluate a female's latency to mating when placed with a single male. Considering that courtship behavior generally contains multimodal signaling, we analyzed 19 behavioral elements demonstrated by both sexes of the cricket *Gryllus bimaculatus* during courtship. To estimate male dominance status, males were preliminarily tested in two rounds of fights. Females mounted males with different fighting ability equally often, but the latencies from the start of antennal contact to mount were shorter in fight losers than fight winners. During courtship, males with high fighting ability demonstrated one of the elements of agonistic display, rocking the body, more frequently, and for longer durations than males with low fighting ability. This element was negatively correlated with singing in fight winners but was positively correlated with singing a courtship song in fight losers. Rocking is thereby suggested to have multiple signaling functions in agonistic and courtship behavior. The song parameters were poorly related with male mating success. Fight winners, rather than fight losers, tended to produce a higher number of calling chirps, which could be explained by the inability of males with high fighting ability to quickly shift from aggression to courtship behavior. The results suggest that increased aggression in fight winners is likely to interfere with subsequent courtship.

Keywords: cricket, *Gryllus bimaculatus*, multimodal signaling, courtship song, dominance status, aggressiveness, female preference

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INTRODUCTION

It is generally thought that intrasexual selection (resulting from male-male competition) and intersexual selection (resulting from female choice) are mutually reinforcing processes (Qvarnstrom and Forsgren, 1998; Wong and Candolin, 2005). In the last quarter of a century, however, various studies have suggested a more intricate relationship between these two processes. Female preference for dominant males has been found in many species (Andersson, 1994; Berglund et al., 1996), in particular, in reptiles (Trivers, 1976), birds (Trail, 1985; Kunc et al., 2006), fishes (Far and Travis, 1986; Bisazza and Marin, 1991), crayfish (Aquiloni et al., 2008), flies (Alcock and Pyle, 1979; Borgia, 1981), and crickets (Simmons, 1986). Female preference for dominant males could have direct benefits if these males provide higher quality resources (Andersson, 1994) or better parental care (Hoelzer, 1989). Female preference for dominant males could also provide indirect benefits if these males sire offspring of superior genetic quality (birds: Norris, 1993; Petrie, 1994; Sheldon et al., 1997; crickets: Wedell and Tregenza, 1999; Bretman et al., 2006).

A growing number of studies, however, demonstrate no correlation between male fighting ability and their attractiveness (Qvarnstrom and Forsgren, 1998). This could occur because dominant males provide less parental care (Forsgren, 1997; Wong, 2004), harm their mates (Moore et al., 2001; Ophir and Galef Jr, 2003), have depleted sperm stores (Pitnick and Markow, 1994; Preston et al., 2001), or are more likely to transmit diseases (Folstad and Karter, 1992). Females could also ignore dominant males because these males might not invest as much in mate attraction or courtship as subordinate males. For example, it was shown in crickets *Teleogryllus oceanicus* that females even preferred subordinate males: rather than investing more in postcopulatory strategies, the subordinate males invested in an alternative precopulatory mating approach (Thomas and Simmons, 2009).

The most common way to investigate female preferences is conducted using simultaneous choice tests. If possible, these tests should exclude male-male interactions; otherwise it would be difficult to evaluate the relationship between intra- and intersexual selection. In crickets, such studies have been conducted using playback experiments (Rantala and Kortet, 2003) or offering filter papers with pheromones of males with different fighting ability (Kortet and Hedrick, 2005). Investigation of long-distance acoustic signals (calling song) could also allow choice tests without male-male interactions (Hedrick and Bunting, 2014).

In choice tests where a female was placed with two male crickets, the winner of the agonistic encounter was more likely to mate (Gryllus bimaculatus: Simmons, 1986; Acheta domesticus: Nelson and Nolen, 1997; Rantala and Kortet, 2004). It was shown that dominant males could prevent subordinate males from courting the females. Because females do not mate with non-courting males (Alexander, 1961), these experiments cannot measure the free choice of females. Moreover, males in the presence of a female were more likely to initiate fights and their fights were more aggressive than in the absence of a female (*G. bimaculatus*: Simmons, 1986; Tachon et al., 1999; *G. veletis*: Fitzsimmons and Bertram, 2013; *G. assimilis*: Montroy et al., 2016). If females prefer to mate with fight winners, thenincreased male aggression might be reinforced by intersexual selection.

A second way to measure female preferences is to conduct no-choice tests. These tests usually measure a female's latency to mating when placed with a single male. In no-choice tests conducted on various species of crickets, the results vary. In *A. domesticus* (Savage et al., 2005) and *G. assimilis* (Loranger and Bertram, 2016), males that win fights were shown to be more attractive to females. By contrast, Nelson and Nolen (1997) and Shackleton et al. (2005) showed that females did not prefer males that won fights in *A. domesticus* and *T. commodus*. In *T. oceanicus,* subordinate males upregulated the quantity of a number of cuticular compounds that increase male mating success; at the same time, they produced ejaculates of lower quality and sired fewer offspring than dominant males (Thomas and Simmons, 2009). Thus, the contact pheromones are not always an honest signal of males' quality, and females may not be able to detect this dishonesty.

Many cricket species display a repertoire that includes three structurally distinct signals, termed the calling (a long-range signal), courtship (a close-range signal) and aggression (produced during encounters with other males) songs (Alexander, 1961). In *G. bimaculatus,* the intensity of calling songs and the repetition rate of chirps and pulses was positively correlated with male size, and larger males gained more matings (Simmons, 1986, 1988). In the field, however, pulse rate was negatively related to male size, while the duration of pulses was positively related to size (Simmons and Zuk, 1992). In *G. integer*, the percentage of time spent singing calling songs was either negatively correlated with aggressiveness (for males caught in the field) or unrelated to aggressiveness (for males raised in the lab) (Hedrick and Bunting, 2014). The parameters of the courtship song have been more poorly investigated in relation to the cricket dominance status. No effect of body size on the dominant frequency of the courtship song was found in *G. bimaculatus* (Miyashita et al., 2016). At the same time, higher rates and durations of ticks (the parameters preferred by females) were positively correlated with high immunocompetence, which may indicate that females might benefit by increasing the parasite resistance of their offspring (Rantala and Kortet, 2003).

In no-choice tests, two parameters of female preferences are usually measured: the percentage of females that mated males and the latency to mating. In the current study, we videotaped male-female interactions and measured many different behavioral elements demonstrated by both sexes in no-choice tests. We hypothesized that males that won fights would behave more aggressively toward females than males that lost fights, and this could be a part of reason decrease mating success of fight winners. Taking into account multisensory courtship signals, we tried to evaluate which sensory modalities, chemical or acoustic, could be a better indicator of the male dominance status. We also analyzed different song parameters to determine whether the songs of dominant and subordinate males differed from each other.

MATERIALS AND METHODS

Crickets

Experimental animals came from a laboratory stock obtained from the Moscow Zoo culture. This culture was originally obtained in 1985–1990 from the cricket farms and pet food stores in Germany and Great Britain. Since this period, the Moscow Zoo culture has been constantly maintained at more than 1,000 individuals. The size of the laboratory stock varied from 30 to 200 individuals at different times; however, this stock was refreshed from the Zoo culture one–two times per year to reduce the potential effects of inbreeding. The crickets were reared in plastic containers (57 × 39 × 42 cm) at 22–27°C under a 12-h:12-h light/dark cycle. Food (dried amphipods and oatflakes) and water were provided *ad libitum*. The crickets were separated into individual containers (12 × 12 × 7 cm) not later than 24 h after the imaginal molt. Thus, individuals were physically but not acoustically isolated. All behavioral experiments were conducted on virgin individuals of one to 2 weeks old under dim red lights in a temperature controlled room (25°C).

Estimating the Male Social Rank

Individual males were ranked for fighting ability by methods similar to those of Shackleton et al. (2005), Savage et al. (2005), and Thomas and Simmons (2009). We tested males in blocks, with four randomly selected males in each block. The age difference of males within each block varied from 0 to 3 days. For discrimination between opponents during contests, males within each block were marked individually on the pronotum with correction permanent markers.

Males were tested in two rounds of fights (**Data Sheet 2**). In the first round, pairs of males were randomly assigned and placed in individual open-top plastic container (15 × 15 × 15 cm). Dominance status was usually established within the first few minutes, when a loser (male with low fighting ability) started to avoid all further aggressive encounters with a winner (male with high fighting ability). This generated two males that won and two males that lost their first round of contests. In the second round, the previous winners were paired (**Video Clip 1**) and the previous losers were paired (**Video Clip 2**). Only those males that lost ($n = 27$) or won ($n = 29$) both contests were used in subsequent experiments with females. The advantage of this method was that every male always competed against another male with the same recent fight history, since the success in previous fights has been shown to increase the likelihood of victory in subsequent fights in crickets (Khazraïe and Campan, 1999; Hofmann and Stevenson, 2000; Savage et al., 2005). By using this method, we also increased the difference between the males with different fighting ability. In all contests, males were left together for a period of 5–7 min. The floor of the arena was covered with a paper towel, which was replaced after each trial to remove any olfactory cues that might be left by the crickets.

Courtship Test Procedure

After the second round of fights (with an interval varied in the range of 1–7 min), we placed a randomly drawn female with the male, one female—with the double winner, and another—with the double loser. The experiments were performed in a cylindrical open-top arena (15 × 15 × 15 cm), in which the floor was covered with a paper towel, and the walls were formed by a metallic grid. We evaluated female preferences based on the readiness of the female to mount the male. During courtship, the male turns away from the female and presents his abdomen, while continuously stridulating. The female reacts by approaching from behind. The male spreads his hind wings and flattens his abdomen, allowing the female to mount him (**Video Clip 3**). Mounting of the male by the female is a prerequisite for copulation (Alexander, 1961; Adamo and Hoy, 1994). A male was introduced into the arena, and after about 1 min we introduced a female. Each trial lasted for up to 7 min. All trials were video recorded (Sony DCR-TRV 355E), and the video signals were transferred to a PC for analysis of courtship. In male–female interactions, each specimen was used only once.

Courtship behavior was analyzed with the BORIS program (Friard and Gamba, 2016). According to this program, the behavior type can be defined as a "state event" and a "point event" (having and not having duration, correspondingly). Overall, we distinguished 15 state and 4 point events in both male and female behavior (**Table 1**). All specific elements during male—female interactions were documented as previously described by Adamo and Hoy (1994). We measured the latency from the test start to the onset of the element (for all events), and the duration of the element (for state events only). We also calculated the latencies from the first antennal contact to male rocking, singing, female following and mount (**Data Sheet 1**).

Simultaneously with video recording, we conducted song recordings. A microphone (type 4191, 1/2 inch; Bruel & Kjær, Nærum, Denmark) was placed at a height of 8–10 cm from the top of the arena. The output of a conditioning amplifier (Bruel and Kjær 2690) was digitized (100 kHz sampling rate) using a custom-made A/D–D/A interface. The temporal parameters and power spectra of the songs were analyzed with COOLEDIT (Syntrillium, Seattle, WA). During courtship tests, males sometimes produced not only a typical courtship song, but also some elements of calling or rivalry (=aggressive) songs (**Figure 1**). A courtship song of *G. bimaculatus* was easy to distinguish from the other two types of the songs: the calling and rivalry songs consisted of chirps containing several pulses; the dominant carrier frequency was ca. 4.5–5.0 kHz (first harmonic). The more variable courtship song was composed of large-amplitude pulses (=ticks) separated by a number of smaller pulses; the energy of ticks was concentrated around 4–5 and 11–16 kHz. The duration of ticks comprised about half of the chirp pulse duration (Rheinlaender et al., 1976; Libersat et al., 1994; Shestakov and Vedenina, 2015). We measured 9 song characters: the ratio of the chirp to tick number, the number of pulses per chirp, the duration and period of the chirp pulses, the dominant frequency of chirps, the duration and period of ticks, the relative amplitude of the courtship pulses and ticks, and the dominant frequency of ticks (**Data Sheet 3**).

RESULTS

The Occurrence of Courtship Elements

Most of the specific elements recorded during male–female interactions occurred in more than 50% of trials (**Figure 2**). Among them, however, only the antennal contact was demonstrated by both sexes in almost all trials. There were no significant differences between males with different fighting ability in the occurrence of any behavioral elements (Fisher's Exact Test, two-tailed; $p > 0.05$), except for rocking ($p = 0.04$): fight winners rocked the body (**Video Clip 4**) more often than fight losers (in 83 vs. 56% of trials). Fight winners also followed females more often (in 69 vs. 44% of trials) and demonstrated pauses less often (in 69 vs. 89% of trials) than fight losers. These differences were, however, not significant ($p = 0.1$). The elements that usually preceded the mounting response (singing, turn, pushing back) occurred in 58–74% of experiments. Females mounted winners and losers almost equally often (in 59 and 67% of trials, respectively; $p = 0.59$).

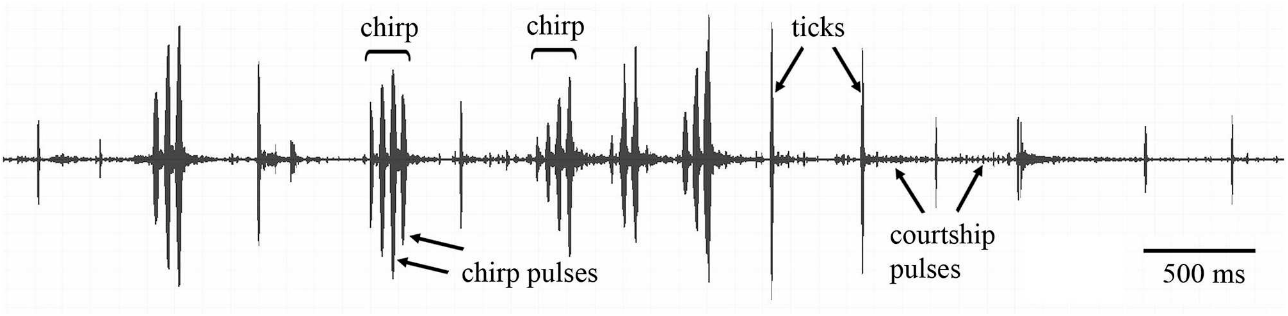

FIGURE 1 | Oscillogram of a song produced during male—female interactions in *Gryllus bimaculatus*. During courtship, the males could produce not only the elements of courtship song (ticks and courtship pulses), but also the elements of calling song (chirps and chirp pulses).

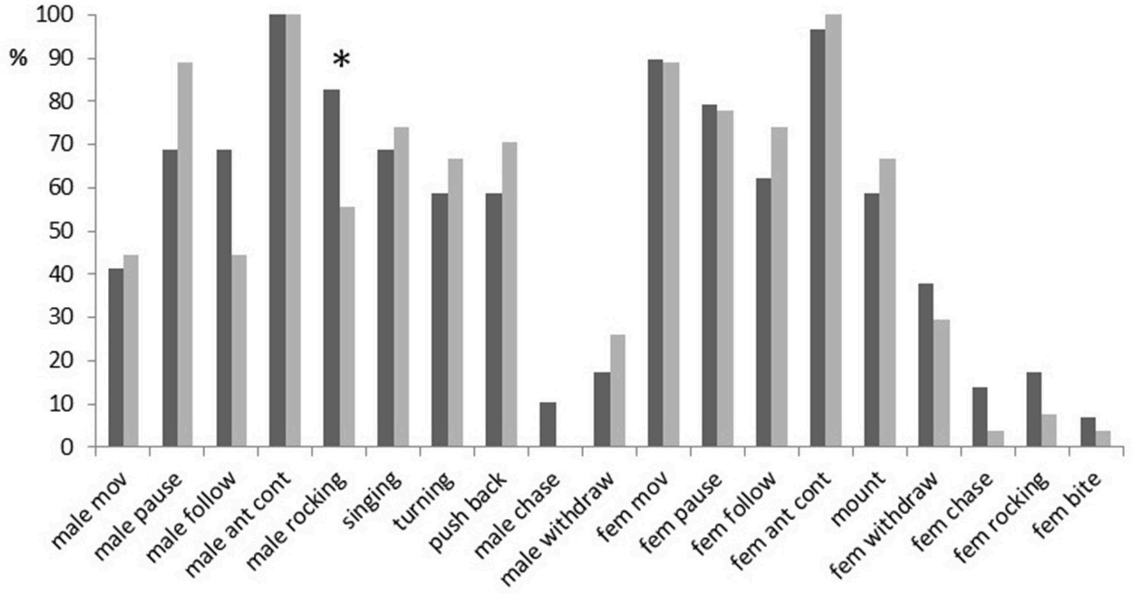

FIGURE 2 | A percentage of trials containing a given behavioral element in courtships of dominant males (black bars) and subordinate males (gray bars) in *Gryllus bimaculatus*. Abbreviations for behavioral elements are listed in **Table 1**. Asterisk indicate statistical significance of the differences between dominant and subordinate males (Fisher's Exact Test, two-tailed; $^*p < 0.05$).

Some elements rarely occurred in male–female interactions. Males with high fighting ability moved away (withdrew) from any contacts with females in 17% of the trials, while males with low fighting ability exhibited this behavior in 26% of the trials. Crickets drove conspecifics out (chased) more often in experiments with fight winners (in 10–14% of cases) than in experiments with fight losers (in 0–4% of trials). Females rocked body and bit dominant males more often (in 17 and 7% of trials, respectively) than subordinate males (in 7 and 4%, respectively). None of these differences were statistically significant ($0.35 < p < 0.5$). We did not analyze latencies or duration of the elements that occurred in <30% of experiments because of the low sampling number.

The Latencies to Courtship Elements

Analysis of the latencies from the onset of experiment to the start of any element showed many differences between winners and losers. Despite these differences were not significant for all elements (after the correction for the false discovery rate; Benjamini and Hochberg, 1995), the consistent patterns can be found in these differences (**Figure 3**). For example, winning males started antennal contact (9 s) and rocking (12 s) earlier than losing males (19 and 32 s, respectively). By contrast, fight winners started to sing, follow females, turn and push back later than fight losers (of median values 53 vs. 31 s; 52 vs. 34 s; 61 vs. 42 s; 109 vs. 60 s, respectively). Females started to withdraw earlier from winning than losing males (32 vs. 62 s), and conversely, started to follow winners later than losers (61 vs.

TABLE 1 | Behavioral elements demonstrated by crickets *Gryllus bimaculatus* during male–female interactions.

Description of behavioral elements	Type of event	Abbreviations used for behavioral elements
Male actively moves for at least ½ body length	State	Male mov
Male remains immobile for at least 10 s	State	Male pause
Male follows female	State	Male follow
Male contacts female with at least one antenna	Point	Male ant cont
Male quickly rocks body back and forth	State	Male rocking
Male stridulates any song type	State	Singing
Male turns to present posterior to female	Point	Turning
Male pushes backwards while female mounts	State	Push back
Male drives female out (male aggression)	State	Male chase
Male moves away from contact with female	State	Male withdraw
Female actively moves for at least ½ body length	State	Fem mov
Female remains immobile for at least 10 s	State	Fem pause
Female follows male	State	Fem follow
Female contacts male with at least one antenna	Point	Fem ant cont
Female walks up onto male's abdomen	Point	Mount
Female moves away from contact with male	Point	Fem withdraw
Female drives male out (female aggression)	State	Fem chase
Female quickly rocks body back and forth	State	Fem rocking
Female pinches male with mandibles	Point	Fem bite

29 s). Females also mount dominant males later than subordinate ones (112 vs. 66 s).

Calculations of the latencies from the onset of antennal contact to the mounting response (**Figure 4**) revealed significant differences between the males with different fighting ability when conducting Mann–Whitney *U*-tests ($p < 0.03$), but not significant differences after the correction for the false discovery rate. The latencies from the onset of male antennal contact to mount were lower in losers (42 s) than winners (89 s). The same was found for the latencies from the onset of female antennal contact to mount (45 s in losers and 89 s in winners).

We found few correlations between the latencies to different courtship elements in males that won fights. The latencies to male and female antennal contacts highly positively correlated (Spearman rank correlation; $r = 0.98$, $p = 0.0000$), and the latency to singing positively correlated with the latencies to pushing back ($r = 0.51$, $p = 0.035$) and female movement ($r = 0.62$, $p = 0.01$). Notably, we did not find any correlations for the latency to rocking in tests with dominant males. In males that lost fights, more correlations between the latencies to different courtship elements were been found. The latency to rocking positively correlated with latencies to singing and turning ($r = 0.86$–0.9, $p < 0.01$). The latency to singing also correlated with latencies to male antennal contact ($r = 0.62$, $p = 0.006$), turning ($r = 0.93$, $p = 0.0000$), pushing back ($r = 0.79$, $p = 0.0000$), following by female ($r = 0.66$, $p = 0.007$), and mount ($r = 0.79$, $p = 0.0000$). All these correlations were only calculated for successful courtships.

The Duration of Courtship Elements

We found the differences in duration of some courtship elements between males with different fighting ability (**Figure 5**), but none of these differences was significant after the correction for the false discovery rate. Fight winners demonstrated longer rocking (7% of all courtship duration), singing (23%) and following of females (8%) than fight losers (2, 14 and 4%, respectively). By contrast, fight winners moved less (5%) than fight losers (11%). Also, females withdrew longer from winning males (8%) than from losing males (4.6%).

We found almost no correlation between the durations of different elements for courtships of fight winners. In successful courtships, two elements only, durations of male rocking and singing, were negatively correlated (Spearman rank correlation; $r = -0.59$, $p = 0.045$). In courtships of fight losers, we found significant positive correlations between the durations of singing and following of females ($r = 0.7$, $p = 0.035$), singing and female movement durations ($r = 0.53$, $p = 0.043$), male movement and female following durations ($r = 0.9$, $p = 0.037$), male and female pause durations ($r = 0.82$, $p = 0.002$). Interestingly, we found a significant negative correlation between the durations of male rocking and mount ($r = -0.83$, $p = 0.01$).

The Songs Produced During Courtship Tests

Almost all males singing in our experiments mated: 17 of 20 singing males that won fights and 18 of 20 singing males that lost fights were successful in courtship. The number of chirps emitted by winners was twice as many as by losers; this difference, however, was not significant (**Table 2**, **Figure 1**). None of the chirp parameters differed between the songs of winners and losers. Ticks, the main element of the courtship song, appeared to differ significantly between the males in dominant frequency, which was higher in dominant than subordinate males. Other parameters of the courtship song were qualitatively but not significantly different between the males. Dominant males tended to produce longer ticks of a shorter period than subordinate males; low-amplitude pulses that alternated with ticks were more prominent in the songs of subordinate than dominant males.

DISCUSSION

Females Mate With Fight Losers Faster Than With Fight Winners

Our results demonstrated that females of *G. bimaculatus* mounted equally often fight winners and fight losers. The latencies from the start of the trial to mount also did not differ in males with different fighting ability. However, the latencies from the start of antennal contact to mount were shorter in males that lost fights. We suggest the latter characteristic to be

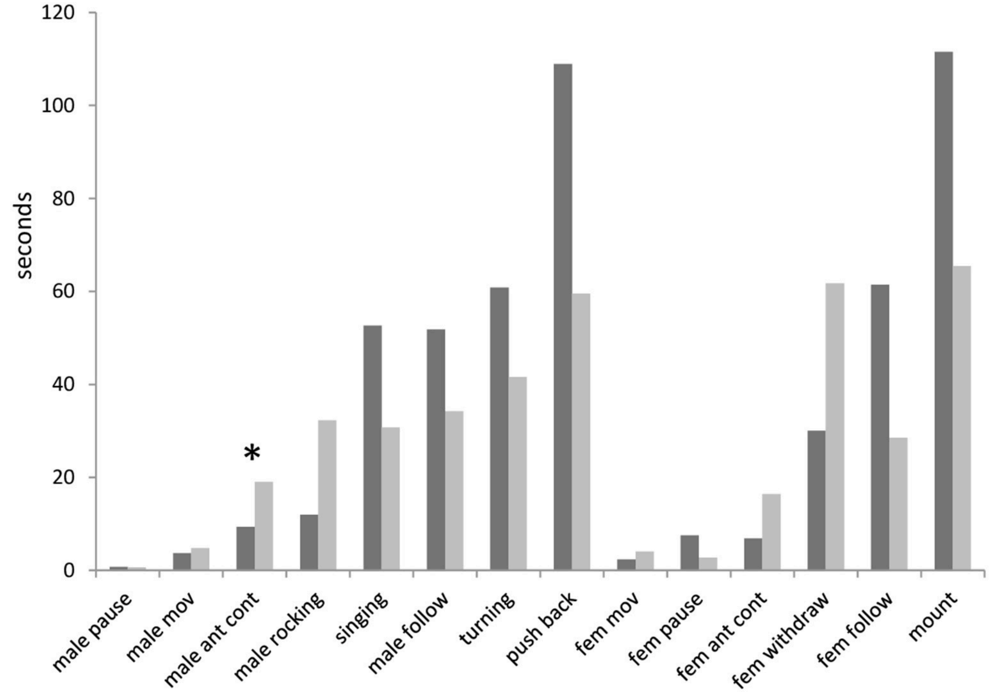

FIGURE 3 | Latencies from the onset of experiment to the start of behavioral elements in courtships of dominant males (black bars) and subordinate males (gray bars) in *Gryllus bimaculatus*. Experiments with successful courtships are only included ($n = 17$ for dominant males and $n = 18$ for subordinate males). Asterisk indicate statistical significance of the differences between dominant and subordinate males (Mann-Whitney *U* Test; $*p < 0.05$).

the important one since the antennal sensory cues are crucial for mounting responses (Loher and Rence, 1978; Adamo and Hoy, 1994). Moreover, contact chemoreception, rather than mechanoreception was shown to be the key modality for mate recognition (Balakrishnan and Pollack, 1997; Tyler et al., 2015).

Our results mainly support the data of Nelson and Nolen (1997) and Shackleton et al. (2005) obtained on *A. domesticus* and *T. commodus,* who found no difference between winners and losers in mating success in no-choice experiments. The shorter latencies to mating in fight losers demonstrated in our tests may also support the results on pheromone expression in *T. oceanicus* (Thomas and Simmons, 2009). Subordinate males of *T. oceanicus* upregulate the quantity of a number of cuticular hydrocarbons (CHC) that increase male mating success. Conversely, dominant males invest less in their pheromone signals but produce ejaculates of higher quality and sire more offspring than subordinate males. Similar results were shown in *Drosophila melanogaster*: females that became very attractive to males by allocating too many CHC resources produced fewer offspring or offspring of lower quality (Wicker and Jallon, 1995; Howard et al., 2003). In crickets, fighting success was shown to be more strongly linked to an increased investment in overall CHC profile rather than to specific CHC blends (Steiger et al., 2013). At the same time, mating success was tightly linked to both a lower investment in overall CHC expression and the higher relative abundance of specific CHC blends (Simmons et al., 2013; Steiger et al., 2015).

Can High Aggressiveness of Males Interfere With Their Motivation to Court?

Fight winners in our tests started to rock their body earlier and demonstrated more frequent and longer rocking than fight losers. The function of rocking, or juddering, is controversial. Rocking is usually suggested to be a component of agonistic display in crickets (Tachon et al., 1999; Bertram et al., 2010). The energetic expense of this display was shown to be of intermediate level, being, however, much higher than during aggressive stridulation (Hack, 1997). Male crickets also rock as a part of their courtship display (Adamo and Hoy, 1994; Vedenina and Pollack, 2012). In different species of arachnids, juddering was shown to signal male quality (Kotiaho, 2000), stimulate females to mate (Briceño and Bonilla, 2009), or serve multiple signaling functions (Gibson and Uetz, 2008). In our experiments, we found a negative correlation between rocking and singing durations in fight winners, but a positive correlation between rocking latency and latencies to singing and turning (that is usually performed by the male to singing the courtship song) in fight losers. We suggest that fight winners demonstrated rocking as a part of agonistic display, whereas fight losers rocked in the context of courtship display. Thus, rocking in *G. bimaculatus* can also be regarded as conveying multiple messages.

Fight winners had a tendency to start singing, follow females, turn and push back later than fight losers. Conceivably the high level of aggressiveness that was retained in winners prevented them from starting a "normal" courtship display. Because of the

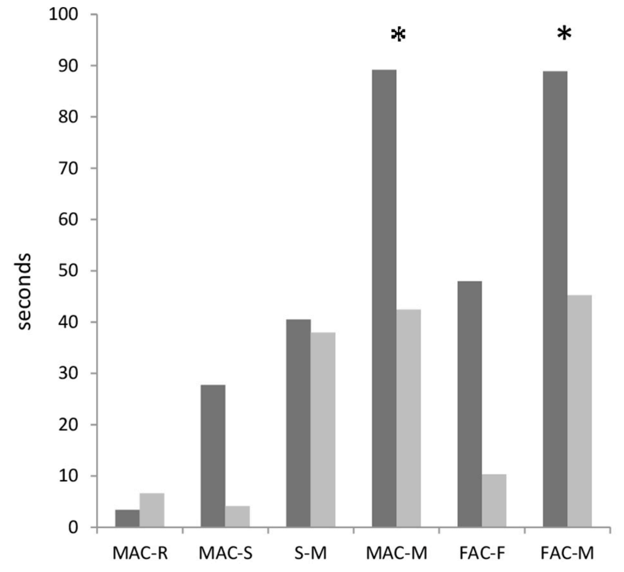

FIGURE 4 | Latencies (median values) from the onset of one to onset of another elements in courtships of dominant males (black bars) and subordinate males (gray bars) in *Gryllus bimaculatus*. Experiments with successful courtships are only included ($n = 17$ for dominant males and $n = 18$ for subordinate males). MAC-R: male antennal contact to rocking; MAC-S: male antennal contact to singing; S-M: singing to mount; FAC-F: female antennal contact to following; FAC-M: female antennal contact to mount. Asterisks indicates statistical significance of the differences between dominant and subordinate males (Mann–Whitney *U*-Test; $^*p < 0.05$).

methods used in our tests, the level of the winner aggressiveness was experimentally set too high. Winners used in the courtship tests won fights in two rounds. We allowed a male to compete against another male with the same recent fight history, since success in previous fights increases the likelihood of victory in subsequent fights in crickets (Khazraïe and Campan, 1999; Savage et al., 2005). In our tests, the second round of fight was usually much more severe than the first round of fight (**Video Clip 1**). To test whether the high level of aggressiveness could interfere with motivation to court, it would be worthwhile to increase the number of fight rounds and study the latencies to singing and other courtship elements after several rounds.

In the wild, males of *G. bimaculatus* tend to find shelters from which they call to attract receptive females (Alexander, 1961; Simmons, 1986). A calling song, however, attracts not only females but also other males; thus, several successive fights with different males could easily happen in nature. If increased aggressiveness would correlate with increasing latency to court females, as could be expected from our tests, this might be maladaptive. Females, however, were more likely to mate with shelter residents: shelters seemed to benefit males by providing protection since calling may attract both parasitoids and predators (Cade, 1975; Simmons, 1986; Robert et al., 1992; Wagner, 1996). Presumably, such a preference of shelter residents allowed a relaxation of selection pressure for a quick shift from aggression to courtship behavior in dominant males.

In contrast to the high level of winner aggressiveness, the level of the loser submissiveness was not set experimentally too low in our tests. The second round of fight was usually much less fierce than the first one (**Video Clip 2**). Thus, the level of readiness to court in losers was closer to that of inexperienced male than the level of such readiness in winners. We suggest that this is part of the reason why fight losers tended to demonstrate the shorter latencies to all main courtship elements. In natural habitats, subordinate males have to be capable of quickly shifting from encounters with another male to courting a female. Subordinate males are unlikely to have shelters which could additionally attract females, and selection pressure acting on male behavioral plasticity through female choice could be stronger in subordinate than dominant males.

A Poor Relationship Between Song Parameters and Male Mating Success

The duration of singing was higher in fight winners than in losers. At the same time, the latencies from the start of singing to mount did not differ significantly between the males with different fighting ability. There were no differences in song parameters between winners and losers except for the difference in dominant frequency of ticks. Ticks (high-amplitude pulses) were found to be a crucial component of a successful courtship song (Libersat et al., 1994). Ticks produced by dominant males were of the higher dominant frequency (11.7 kHz) than ticks generated by subordinate males (9.7 kHz). In playback experiments, however, synthesized songs with different carrier frequencies of ticks (varied from 5 to 17 kHz) were as attractive to females as courtship of muted males accompanied by playback of the recorded song (Shestakov and Vedenina, 2015). Thus, the difference in tick dominant frequency between winners and losers was unlikely to influence female preferences but it might reflect differences in body size of males. As it was shown in many animals, both vertebrates and invertebrates, smaller individuals tend to produce higher-frequency calls due to resonant cavities and muscular rate contractions scaling with body size (e.g., Bailey, 1970; Wallschager, 1980; Brown et al., 1996). The lower dominant frequency of ticks shown in fight losers could indicate that they were on average larger than the fight winners. This seems contrary to the results in several studies obtained earlier on different cricket species (Simmons, 1986; Savage et al., 2005; Shackleton et al., 2005), which demonstrated that larger males won more fights. Hofmann and Schildberger (2001), however, found that weight asymmetry was not a very reliable predictor of outcome, duration, or intensity of fights between two males of *G. bimaculatus*. We measured neither weight nor body length, but noticed that lighter males sometimes won fights even when the weight asymmetry was large.

Female crickets prefer courtship songs with a long duration of ticks (Rantala and Kortet, 2003). In our tests, tick duration tended to be higher in the songs of winners, which were definitely not preferred by females. Our results seem to match the data of our previous study, in which increasing the duration of ticks had a crucial effect on female response rate, decreasing female responsiveness (Shestakov and Vedenina, 2015).

The number of chirps emitted by winners was twice as many as that emitted by losers. Despite this difference was non-significant, we suggest that a tendency to produce a higher number of chirps by dominant males in the presence of a female could be also explained by their inability to quickly shift

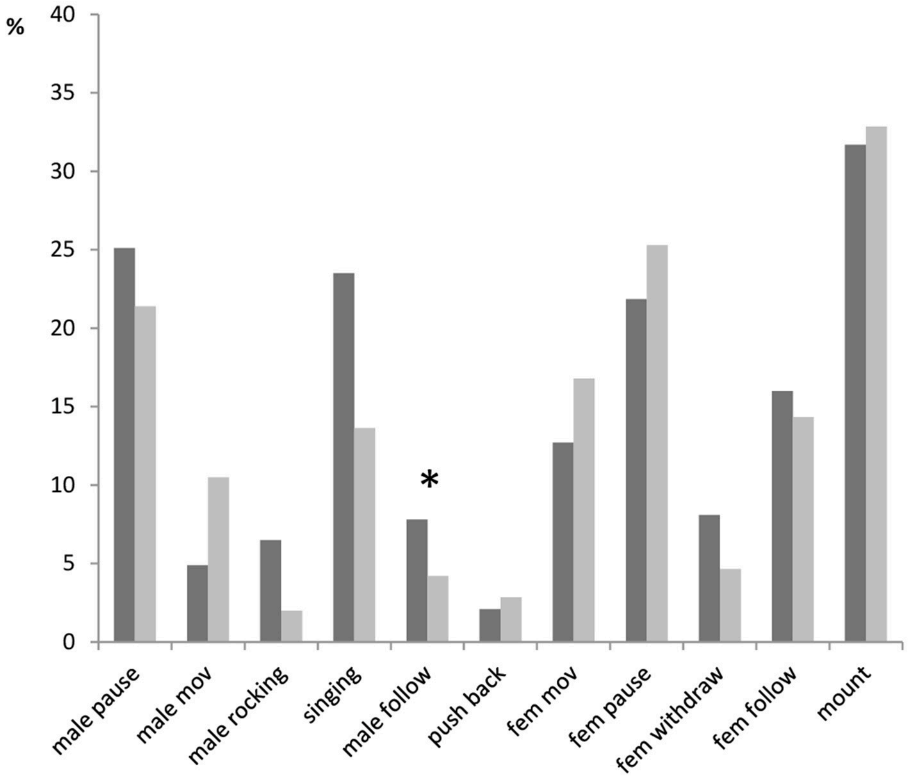

FIGURE 5 | Relative duration (median value) of behavioral elements in courtships of dominant males (black bars) and subordinate males (gray bars) in *Gryllus bimaculatus*. Experiments with successful courtships are only included ($n = 17$ for dominant males and $n = 18$ for subordinate males). Asterisk indicate statistical significance of the differences between dominant and subordinate males (Mann–Whitney *U*-Test; $^*p < 0.05$).

TABLE 2 | The values of the song parameters produced during male-female interactions in *Gryllus bimaculatus* by dominant and subordinate males, and statistics of comparisons between the two male groups.

Parameter	Dominant males	Subordinate males	*T*-test	Mann–Whitney *U*-Test
The number of chirps in relation to ticks, %	37.55 (1.28; 66.19)	15.24 (0; 43.33)	n/a	$U = 170$; $p = 0.14$
Chirp pulse duration, ms	21.99 ± 3.21	20.21 ± 4.02	$t = 1.27$, df = 25, $p = 0.22$	n/a
Chirp pulse period, ms	39.37 ± 5.23	38.36 ± 5.95	$t = 0.46$, df = 25, $p = 0.65$	n/a
Pulse number per chirp	3.59 ± 1.26	3.11 ± 0.54	$t = 1.15$, df = 25, $p = 0.26$	n/a
Chirp dominant frequency, kHz	4.914 ± 0.179	4.934 ± 0.284	$t = -0.23$, df = 25, $p = 0.82$	n/a
Tick duration, ms	11.60 ± 1.93	10.04 ± 3.22	$t = 1.78$, df = 36, $p = 0.08$	n/a
Tick period, ms	346.94 ± 89.27	424.13 ± 314.19	$t = -1.01$, df = 36, $p = 0.32$	n/a
Tick dominant frequency, kHz	11.691 ± 2.041	9.687 ± 2.944	$t = 2.41$, df = 36, $p = 0.02$	n/a
Courtship pulse to tick amplitude	0.057 ± 0.033	0.078 ± 0.073	$t = -1.12$, df = 36, $p = 0.27$	n/a

The median values and lower/upper quartiles are shown for the first parameter; the mean and standard deviation are presented for other parameters.

from aggression to courtship behavior. Because of the relatively low number of pulses per chirp (3-4), we suggest they belong basically to the calling but not to the rivalry song (Zhantiev and Dubrovin, 1974; Rheinlaender et al., 1976). In any case, singing of the calling or rivalry song nearby a female could signal to a female that the male is not ready to copulate. Fight winners thereby appeared to be less ready to mate than fight losers.

Perspectives

Our analysis showed that subordinate males demonstrated shorter latencies from antennal contact to mount as compared with dominant males. This result is not consistent with the traditional view that females should prefer males with increased fighting ability. Using the method of the two-round fights we enhanced the level of aggressiveness in fight winners, thus increasing the difference between winners and losers. As a result, during the male—female interactions, fight winners demonstrated one of the elements of agonistic display, i.e., rocking body, more frequently, and for longer durations than the fight losers, and this behavior seemed to interfere with subsequent courtship. Future research should investigate whether an increase of the fight round number would lead to slower latencies to singing or other courtship elements. It is also possible that the high level of aggressiveness might inhibit the immediate shift to courtship behavior; however, this inhibition may disappear with increasing the delay time from the last fight to courtship. It was shown in *G. bimaculatus* that the aggressive behavior of males was influenced by prior agonistic experience for 6 h and the effect disappeared entirely after 24 h (Khazraïe and Campan, 1999). The submissive behavior was also shown in subordinate males for at least 3 h (Stevenson and Rillich, 2016). Future studies that estimate the influence of aggressiveness on courtship success depending on the delay time after fight could provide insights into our understanding of the physiological mechanisms underlying such behavior.

ETHICS STATEMENT

All animal handling and behavior sampling methods followed the quidelines of the Institutional Animal Ethics Committee and complied with the laws of Russia.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

VV and LS were equally involved in design of the study, conducting the experiments and performing statistical analyses. VV wrote 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.2018.00197/full#supplementary-material

Data Sheet 1 | Latencies and durations of courtship elements.

Data Sheet 2 | Experimental protocol of fights and courtships.

Data Sheet 3 | Courtship song measurements.

Video Clip 1 | Second round of fight between previous winners.

Video Clip 2 | Second round of fight between previous losers.

Video Clip 3 | Courtship and copulation.

Video Clip 4 | Rocking the body by fight winner.

REFERENCES

Adamo, S. A., and Hoy, R. R. (1994). Mating behavior of the field cricket *Gryllus bimaculatus* and its dependence on social and environmental cues. *Anim. Behav*. 47, 857–868. doi: 10.1006/anbe.1994.1117

Alcock, J., and Pyle, D. W. (1979). The Complex courtship behavior of *Physiphora demandata* (F.) (Diptera: Otitidae). *Ethology* 49, 352–362.

Alexander, R. D. (1961). Aggressiveness, territoriality, and sexual behavior in field crickets (Orthoptera: Gryllidae). *Behaviour* 17, 130–223. doi: 10.1163/156853961X00042

Andersson, M. (1994). *Sexual Selection. Princeton, NJ: Princeton University Press.*

Aquiloni, A., Burič, M., and Gherardi, F. (2008). Crayfish females eavesdrop on fighting males before choosing the dominant mate. *Curr. Biol*. 18, R462–R463. doi: 10.1016/j.cub.2008.04.006

Bailey, W. J. (1970). The mechanics of stridulation in bush crickets (Tettigonioidea, Orthoptera) I. Tegminal Generator. *J. Exp*. Biol. 52, 495–505.

Balakrishnan, R., and Pollack, G. (1997). The role of antennal sensory cues in female responses to courting males in the cricket *Teleogryllus oceanicus*. *J. Exp. Biol*. 200, 511–522.

Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. Royal Stat. Soc. B* 57, 289–300.

Berglund, A., Bisazza, A., and Pilastro, A. (1996). Armaments and ornaments: an evolutionary explanation of traits of dual utility. *Biol. J. Linnean Soc*. 58, 385–399. doi: 10.1111/j.1095-8312.1996.tb01442.x

Bertram, S. M., Rook, V. L. M., and Fitzsimmons, L.P. (2010). Strutting their stuff: victory displays in the spring field cricket, *Gryllus veletis*. *Behaviour* 147, 1249–1266. doi: 10.1163/000579510X514535

Bisazza, A., and Marin, G. (1991). Male size and female mate choice in the eastern mosquitofish *(Gambusia holbrooki*: Poeciliidae). *Copeia* 3, 730–735. doi: 10.2307/1446400

Borgia, G. (1981). Mate selection in the fly *Scatophaga stercoraria*: female choice in a male-controlled system. *Anim. Behav*. 29, 71–80.

Bretman, A., Rodríguez-Muñoz, R., and Tregenza, T. (2006). Male dominance determines female egg laying rate in crickets. *Biol. Lett*. 2, 409–411. doi: 10.1098/rsbl.2006.0493

Briceño, R. D., and Bonilla, F. (2009). Substrate vibrations in the scorpion *Centruroides margaritatus* (Scorpiones: Buthidae) during courtship. *Rev. Biol. Trop*. 57, 267–274.

Brown, W. D., Wideman, J., Andrade, M. C. B., Mason, A. C., and Gwynne, D. T. (1996). Female choice for an indicator of male size in the song of the black-horned tree cricket *Oecanthus nigricornis* (Orthoptera: Gryllidae: Oecanthinae). *Evol*. 50, 2400–2411. doi: 10.1111/j.1558-5646.1996.tb03627.x

Cade, W. H. (1975). Acousucally orienting parasitoids: fly phonotaxis to cricket song. *Science* 190, 1312–1313. doi: 10.1126/science.190.4221.1312

Far, J. A., and Travis, J. (1986). Fertility advertisement by female sailfin mollies, *Poecilia latipinna* (Pisces: Poeciliidae). *Copeia* 2, 467–472. doi: 10.2307/1445004

Fitzsimmons, L. P., and Bertram, S. M. (2013). Signaling effort does not predict aggressiveness in male spring field crickets. *Behav. Ecol. Sociobiol*. 67, 213–220. doi: 10.1007/s00265-012-1441-1

Folstad, I., and Karter, A. J. (1992). Parasites, bright males, and the immunocompetence handicap. *Am. Nat.* 139, 603–622.

Forsgren, E. (1997). Female sand gobies prefer good fathers over dominant males. *Proc. R. Soc. Lond. B* 264, 1283–1286.

Friard, O., and Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods Ecol. Evol.* 7, 1325–1330. doi: 10.1111/2041-210X.12584

Gibson, J. S., and Uetz, G. W. (2008). Seismic communication and mate choice in wolf spiders: components of male seismic signals and mating success. *Anim. Behav*. 75, 1253–1262. doi: 10.1016/j.anbehav.2007.09.026

Hack, M. A. (1997). Assessment strategies in the contests of male crickets, *Acheta domesticus* (L.). *Anim. Behav*. 53, 733–747. doi: 10.1006/anbe.1996.0310

Hedrick, A., and Bunting, J. (2014). An attractive male trait and aggressiveness are negatively correlated in wild field crickets, but uncorrelated in lab-reared crickets. *Behav. Ecol. Sociobiol*. 68, 233–238. doi: 10.1007/s00265-013-1638-y

Hoelzer, G. A. (1989).The good parent process of sexual selection. *Anim. Behav*. 38, 1067–1078. doi: 10.1016/S0003-3472(89)80146-0

Hofmann, H. A., and Schildberger, K. (2001). Assessment of strength and willingness to fight during aggressive encounters in crickets. *Anim. Behav*. 62, 337–348. doi: 10.1006/anbe.2001.1746

Hofmann, H. A., and Stevenson, P. A. (2000). Flight restores fight in crickets. *Nature* 403:613. doi: 10.1038/35001137

Howard, R. W., Jackson, L. L., Banse, H., and Blows, M. W. (2003). Cuticular hydrocarbons of *Drosophila birchii* and *D. serrata*: identification and role in mate choice in *D. serrata*. *J. Chem. Ecol*. 29, 961–976. doi: 10.1023/A:1022992002239

Khazraïe, K., and Campan, M. (1999). The role of prior agonistic experience in dominance relationships in male crickets *Gryllus bimaculatus* (Orthoptera: Gryllidae). *Behav. Process.* 44, 341–348. doi: 10.1016/S0376-6357(98)00058-8

Kortet, R., and Hedrick, A. (2005). The scent of dominance: female field crickets use odour to predict the outcome of male competition. *Behav. Ecol. Sociobiol*. 59, 77–83. doi: 10.1007/s00265-005-0011-1

Kotiaho, J. S. (2000). Testing the assumptions of conditional handicap theory: costs and condition dependence of a sexually selected trait. *Behav. Ecol. Sociobiol*. 48, 188–194. doi: 10.1007/s002650000221

Kunc, H. P., Amrhein, V., and Naguib, M. (2006). Vocal interactions in nightingales, *Luscinia megarhynchos*: more aggressive males have higher pairing success. *Animal Behav*. 72:25e30. doi: 10.1016/j.anbehav.2005.08.014

Libersat, F., Murray, J. A., and Hoy, R. R. (1994). Frequency as a releaser in the courtship song of two crickets, *Gryllus bimaculatus* (de Geer) and *Teleogryllus oceanicus*: a neuroethological analysis. *J. Comp. Physiol. A*. 174, 485–494. doi: 10.1007/BF00191714

Loher, W., and Rence, B. (1978). The Mating behavior of *Teleogryllus commodus* (Walker) and its central and peripheral control. *Ethology* 46, 225–259.

Loranger, M. J., and Bertram, S. M. (2016). The effect of male dominance on female choice in a field cricket (*Gryllus assimilis*). *Anim. Behav*. 114, 45–52. doi: 10.1016/j.anbehav.2016.01.020

Miyashita, A., Kizaki, H., Sekimizu, K., and Kaito, C. (2016). No effect of body size on the frequency of calling and courtship song in the two-spotted cricket, *Gryllus bimaculatus. PLoS ONE* 11:e0146999. doi: 10.1371/journal.pone.0146999

Montroy, K., , Loranger, M. J., and Bertram, S. M. (2016). Male crickets adjust their aggressive behavior when a female is present. *Behav. Process* 124, 108–114. doi: 10.1016/j.beproc.2015

Moore, A. J., Gowaty, P. A., Wallin, W. G., and Moore, P. J. (2001). Sexual conflict and the evolution of female mate choice and male social dominance. *Proc. R. Sci.* 268, 517–523. doi: 10.1098/rspb.2000.1399

Nelson, C. M., and Nolen, T. G. (1997). Courtship song, male agonistic encounters, and female mate choice in the house cricket, *Acheta domesticus* (Orthoptera: Gryllidae). *J. Insect. Behav*. 10, 557–570. doi: 10.1007/BF02765377

Norris, R. (1993). Heritable variation in a plumage indicator of viability in male great tits *Parus major. Nature* 362, 537–539.

Ophir, A. G., and Galef Jr, B. G. (2003). Female Japanese quail that 'eavesdrop' on fighting males prefer losers to winners. *Anim. Behav*. 66, 399–407. doi: 10.1006/anbe.2003.2230

Petrie, M. (1994). Improved growth and survival of offspring of peacocks with more elaborate strains. *Nature* 371, 598–599. doi: 10.1038/371598a0

Pitnick, S., and Markow, T. A. (1994). Male gametic strategies: sperm size, testes size, and the allocation of ejaculate among successive mates by the sperm-limited fly Drosophila pachea and its relatives. *Am. Nat.* 143, 785–819.

Preston, B. T., Stevenson, I. R., Pemberton, J. M., and Wilson, K. (2001). Dominant rams lose out by sperm depletion. *Nature* 409, 681–682. doi: 10.1038/35055617

Qvarnstrom, A., and Forsgren, E. (1998). Should females prefer dominant males? *TREE* 13, 498–501. doi: 10.1016/S0169-5347(98)01513-4

Rantala, M.J., and Kortet, R. (2003). Courtship song and immune function in the field cricket *Gryllus bimaculatus. Biol. J. Linn. Soc.* 79, 503–510. doi: 10.1046/j.1095-8312.2003.00202.x

Rantala, M. J., and Kortet, R. (2004). Male dominance and immunocompetence in a field cricket. *Behav. Ecol.* 15, 187–191. doi: 10.1093/beheco/arg103

Rheinlaender, J., Kalmring, K., Popov, A. V., and Rehbein, H. (1976). Brain projections and information processing of biologically significant sounds by two large ventral-cord neurons of *Gryllus bimaculatus* DeGeer (Orthoptera, Gryllidae). *J. Comp. Physiol*. 110, 251–269. doi: 10.1007/BF00659143

Robert, D., Amoroso, J., and Hoy, R. R. (1992). The evolutionary convergence of hearing in a parasitoid fly and its cricket host. *Science* 258, 1135–1137. doi: 10.1126/science.1439820

Savage, K. E., Hunt, J., Jennions, M. D., and Brooks, R. (2005). Male attractiveness covaries with fighting ability but not with prior fight outcome in house crickets. *Behav. Ecol*. 16, 196–200. doi: 10.1093/beheco/arh143

Shackleton, M. A., Jennions, M. D., and Hunt, J. (2005). Fighting success and attractiveness as predictors of male mating success in the black field cricket, *Teleogryllus commodus*: the effectiveness of no-choice tests. *Behav. Ecol. Sociobiol*. 58, 1–8. doi: 10.1007/s00265-004-0907-1

Sheldon, B. C., Merilö, J., Qvarnström, A., Gustafsson, L., and Ellegren, H. (1997). Paternal genetic contribution to offspring condition predicted by size of male secondary sexual character. *Proc. R. Soc. Lond. B* 264, 297–302.

Shestakov, L. S., and Vedenina, Yu. V. (2015). Broad selectivity for courtship song in the cricket *Gryllus bimaculatus*. *Ethology* 121, 966–976. doi: 10.1111/eth.12409

Simmons, L. W. (1986). Inter-male competition and mating success in the field cricket, *Gryllus bimaculatus* (de Geer). *Anim. Behav*. 34, 567–579.

Simmons, L. W. (1988). The contribution of multiple mating and spermatophore consumption to the lifetime reproductive success of female field crickets (*Gryllus bimaculatus*). *Ecol. Entomol*. 13, 57–69.

Simmons, L. W., Thomas, M. L., Simmons, F. W., and Zuk, M. (2013). Female preferences for acoustic and olfactory signals during courtship: male crickets send multiple messages. *Behav. Ecol*. 24, 1099–1107. doi: 10.1093/beheco/art036

Simmons, L. W., and Zuk, M. (1992). Variability in call structure and pairing success of male field crickets, *Gryllus bimaculatus*: the effects of age, size and parasite load. *Anim. Behav*. 44, 1145–1152.

Steiger, S., Capodeanu-Nägler, A., Gershman, S. N., Weddle, C. B., Rapkin, J., Sakaluk, S. K., et al. (2015). Female choice for male cuticular hydrocarbon profile in decorated crickets is not based on similarity to their own profile. *J. Evol. Biol*. 28, 2175–2186. doi: 10.1111/jeb.12740

Steiger, S., Ower, G. D., Stökl, J., Mitchell, C., Hunt, J., and Sakaluk, S. K. (2013). Sexual selection on cuticular hydrocarbons of male sagebrush crickets in the wild. *Proc. R. Soc. B* 280:20132353. doi: 10.1098/rspb.2013.2353

Stevenson, P. A., and Rillich, J. (2016). Controlling the decision to fight or flee: the roles of biogenic amines and nitric oxide in the cricket. *Curr. Zool.* 62, 265–275. doi: 10.1093/cz/zow028r

Tachon, G., Murray, A. M., Gray, D. A., and Cade, W. H. (1999). Agonistic displays and the benefits of fighting in the field cricket, *Gryllus bimaculatus. J. Insect Behav*. 12, 533–543.

Thomas, M. L., and Simmons, L. W. (2009). Male dominance influences pheromone expression, ejaculate quality, and fertilization success in the Australian field cricket, *Teleogryllus oceanicus*. *Behav. Ecol.* 20, 1118–1124. doi: 10.1093/beheco/arp105

Trail, P. W. (1985). Courtship disruption modifies mate choice in a lek-breeding bird. *Science* 227, 778–779. doi: 10.1126/science.227.4688.778

Trivers, R. L. (1976). Sexual selection and resource-accruing abilities in *Anoils garmani*. *Evolution* 30, 253–269. doi: 10.1111/j.1558-5646.1976.tb00908.x

Tyler, F., Fisher, D., d'Ettorre, P., Rodríguez-Muñoz, R., and Tregenza, T. (2015). Chemical cues mediate species recognition in field crickets. *Front. Ecol. Evol.* 3:48. doi: 10.3389/fevo.2015.00048

Vedenina, V. Y., and Pollack, G. S. (2012). Recognition of variable courtship song in the field cricket *Gryllus assimilis*. *J. Exp. Biol.* 215, 2210–2219. doi: 10.1242/jeb.068429

Wagner, Jr. W. E. (1996). Convergent song preferences between female field crickets and acoustically orienting parasitoid flies. *Behav. Ecol.* 7, 279–285. doi: 10.1093/beheco/7.3.279

Wallschager, D. (1980). Correlation of sound frequency and body weight in passerine birds. *Experientia (Basel)* 36:412. doi: 10.1007/BF01975119

Wedell, N., and Tregenza, T. (1999). Ssuccessful fathers sire successful sons. *Evolution* 53, 620–625. doi: 10.1111/j.1558-5646.1999.tb03797.x

Wicker, C., and Jallon, J. M. (1995). Influence of ovary and ecdysteroids on pheromone biosynthesis in *Drosophila melanogaster* (Diptera: Drosophilidae). *Eur. J. Entomol.* 92, 197–202.

Wong, B. B. M. (2004). Superior fighters make mediocre fathers in the Pacific blue-eye fish. *Anim. Behav.* 67, 583–590. doi: 10.1016/j.anbehav.2003.08.015

Wong, B. B. M., and Candolin, U. (2005). How is female mate choice affected by male competition? *Biol. Rev.* 80, 559–571. doi: 10.1017/S1464793105006809

Zhantiev, R. D., and Dubrovin, N. N. (1974). Acoustic signals of crickets (Orthoptera, Oecanthidae, Gryllidae). [in Russian] zool. *J.* 53, 345–358.

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REVIEW
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The Role of Learning on Insect and Spider Sexual Behaviors, Sexual Trait Evolution, and Speciation

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We review experimental and theoretical evidence that learning in insects and spiders affects the expression of mate preferences and of sexual signals, the evolution of both traits, and ultimately patterns of assortative mating, and speciation. Both males and females can modify their sexual preferences and signaling based on previous social interactions or the experience of visual, olfactory, gustatory, or auditory signals. Learning takes place during an early life exposure, previous personal sexual experiences or by observing the choices of others, and it can occur sometimes via very short (a few seconds) exposures to individuals or signals. We briefly review some of the molecular mechanisms that mediate learning in insects, as well as theoretical work that assesses how learning impacts the evolution of insect sexual traits and speciation. We suggest that future research should attempt to provide evidence of the adaptive nature of learning, which remains scarce in insects as well as in vertebrates, and explore further the mechanisms of learning in order to probe into their possible transgenerational inheritance. Future studies should also model how this process might further affect the evolution of sexual traits, and provide a unifying terminology for the underlying mechanisms of learning across diverse life-history contexts.

Keywords: mate choice, preference, selectivity, signal, social experience, adaptive value, sexual selection

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INTRODUCTION

Sexual behaviors such as the expression of a mate preference or the expression of a sexual signal are often not fixed but can be modified through social experience in both vertebrates and invertebrates. This leads to animals learning a mate preference or learning to display a sexual signal such as a courtship dance or the release of a pheromone blend. Learning sexual behaviors has been accepted for quite some time in mammals and birds, where most research has been conducted (Hebets and Sullivan-Beckers, 2010; Verzijden et al., 2012; Morand-Ferron and Quinn, 2015; Servedio, 2015; Head et al., 2016), including early work by Konrad Lorenz on learned sexual preferences via imprinting. However, learning in sexual selection remained controversial for insects and other arthropods until recently (Dukas, 2006, 2008a). This stems from insects being thought of as having fixed sexual behaviors due to their short lives and few mating opportunities, limiting their possibilities for learning or its likely adaptive value. However, a large number of more recent studies have illustrated that both insects and spiders modify their behavioral sexual interactions upon previous experience. Furthermore, it is now abundantly clear that many species of insects mate multiply, and have complex brain structures allowing short and long term memory of previous experiences that impact their lifetime mating behavior (Dukas, 2006, 2008a; Chittka and Niven, 2009).

The effect of social experiences, or simply the exposure to a sexual signal such as a pheromone, on the expression of mate preferences and sexual signals has been described in different terms by different authors (**Tables 1**, **2**), from "learning" and "courtship conditioning," to "mate copying," to "exposure," to "premating interaction," "social learning," "experience," "eavesdropping," "mate preference learning," and "learned mate recognition," among a few. This diverse terminology reflects a burgeoning field for insects and spiders over the last decade, as well as the diversity of learning mechanisms that may be at work in these animals. Regardless of terminology (**Table 3**), what all these cases have in common is a significant change in the expression of mating behaviors that results from a prior social experience or previous exposure to a sexual signal.

Our goals in this review are to: (1) highlight the multiple types of information that insects learn that later result in changes in their mating preferences or in the expression of their sexual signals; (2) propose a systematic categorization of the underlying learning mechanisms, by building on a framework developed in vertebrates (**Table 3**); (3) review some of the molecular mechanisms underlying the learning process; (4) review some of the relevant mathematical models, originally applied to vertebrates, that suggest that learning mechanisms have consequences to the evolution of sexual traits and reproductive isolation in insects and spiders. We end by suggesting opportunities for future research in this field.

INSECTS AND SPIDERS LEARN A VARIETY OF SOCIAL INFORMATION AND SIGNALS THAT IMPACT THEIR MATING BEHAVIOR

Learning in the context of mating has been described in a large diversity of spider and insect species (**Tables 1**, **2**). Multiple definitions of learning were used across disciplines, but here, we define learning broadly as being a change in the future sexual behavior of an individual resulting from a previous social experience (Thorpe, 1963; Barron et al., 2015). This definition encompasses all described processes of learning (**Table 3**), for which the adaptive value of the changed behavior has usually not been demonstrated. Learning often involves changes to mating preferences or to speed of sexual response in females, while it usually involves changes in the level of expression of sexual signals in males. In the majority of investigated cases, and perhaps contrary to what is often assumed, naïve females often do not develop a sexual preference until they are exposed to other members of their species or of closely related species (**Figure 1A**). The absence of a naïve preference appears to be especially common for visual sexual signals, which have been the topic of most research. However, when an innate sexual preference is observed, females can also modify it (**Figure 1B**) or become more selective (narrow their preference regarding potential mates) (**Figure 1C**). In males, learning can lead to changes in the courtship intensity, latency to court, target of courtship, or sometimes the expression of the sexual signals.

Most experimental evidence of learning in altering sexual behavior in insects comes from early exposure of sexually immature adults to the phenotypes of surrounding individuals, what has been called "sexual imprinting" in vertebrates. However, sexual behaviors can also vary after the observation of the interaction between other mating individuals, such as in cases of mate-choice copying (e.g., Mery et al., 2009) or imitation of sexual signaling (e.g., Clark et al., 2012, 2015), or from previous sexual interactions with or without mating (**Tables 1–3**). Most studies do not demonstrate that male or female behavioral shifts have an adaptive value, but such value is often assumed. Below, we illustrate this growing experimental evidence of learning in arthropods organized by the type of learned information: social information with unidentified individual signals (when the full phenotype of the interacting individuals is provided to the "learner"), or of specific signals such as olfactory, tactile, visual, and acoustic including vibratory signals (see **Tables 1**, **2** for a more complete overview, **Figure 2**).

Social Exposure Without Clearly Identified Learned Signals

Authors report that a social experience, with or without mating, affects subsequent sexual interactions of focal individuals. In most of these studies, adults are exposed to the full phenotype of other individuals where the specific assessed and learned signal(s) are not clearly identified. For example, virgin female crickets reared in isolation approach and contact males less frequently than virgin females reared in a group (Tinghitella, 2014). Being housed with groups of males or females induces *fruitless* knockout *Drosophila* males, which have lost their ability to court, to recover their courtship behavior, and wild type males to reduce their same-sex sexual behaviors (Bailey et al., 2013; Pan and Baker, 2014). Burrow-digging spider males enlarged their burrows upon rejections by females, which increases their chances of mating, as females prefer larger burrows (Carballo et al., 2017).

The mating status or novelty of the interacting individuals appears to be particularly important in modifying a focal individual's subsequent behavior. For instance, naive female crickets and spiders are more likely to mate, copulate more quickly or cannibalize fewer males than their mated counterparts (Johnson, 2005; Wilder and Rypstra, 2008; Judge et al., 2010). Male fruit flies learn to focus their courtship toward receptive conspecific females, and out-compete sexually inexperienced males, based on previous copulations (Saleem et al., 2014), or based on rejections by mated or virgin females (Dukas, 2005; Ejima et al., 2005; Griffith and Ejima, 2009). These males also learn from rejections from heterospecific females (Dukas, 2004, 2006, 2008b, 2009; Kujtan and Dukas, 2009; Dukas and Dukas, 2012; Dukas and Baxter, 2014) or immature males (Gailey et al., 1982; McRobert and Tompkins, 1988; Bretman et al., 2010). Learning to quickly discriminate receptive from unreceptive individuals is likely adaptive as males can reduce the costs of unsuccessful courting and mating with an unreceptive female or

TABLE 1 | A non-exhaustive list of publications about how male insects and spiders learn sexual behaviors.

Common and species names	Type of learning (as per the authors)	Learned signal and methodology	Altered behavior	References
Fruit fly *Drosophila melanogaster*	Courtship conditioning and associative learning	Full phenotype and olfactory cues. Adult males trained with one or more mated females for an hour. Male courtship latency and proportion of males that court measured a few minutes after training.	Previous rejections by mated females increase the time males spent courting and reduced courtship latency toward sexually receptive females. Rejections are associated to cVA hydrocarbons.	Dukas, 2005 Ejima et al., 2005 Ejima et al., 2007
Fruit fly *Drosophila melanogaster*	Learning from experience Social learning	Full phenotype. Training: 5-day-old virgin males courted only, or with interrupted copulation, or with copulation with a naïve female. Proportion of time courting (relative to the copulation latency) measured ∼45 min after training.	Sexually experienced males (including copulation) spent significantly less time courting and achieved more copulation than sexually naive males.	Saleem et al., 2014
Fruit flies *D. melanogaster* *D. persimilis* *D. pseudoobscura*	Courtship conditioning Learning	Full phenotype. Training: 1-day-old and 4-day-old males courted females for several durations. Duration, latency and proportion of courtship toward con- or heterospecific measured from a few minutes to an hour after training.	Males increased the time spent courting and reduced the courtship latency toward conspecific females.	Dukas, 2004 Dukas, 2008b Dukas, 2009 Kujtan and Dukas, 2009 Dukas and Dukas, 2012 Dukas and Baxter, 2014
Fruit flies *D. melanogaster* *D. affinis*	Courtship conditioning Learning	Full phenotype. Training: young and old males housed with males or females at different densities, for various durations and frequencies. Proportion of time courting females and copulation duration measured shortly after training.	Males rejected by immature males, and immature males receiving courtship by other males, increased their time courting virgin females, increased their copulation duration and reduced their mating latency (compared to solitary males).	Gailey et al., 1982 McRobert and Tompkins, 1988 Bretman et al., 2010 Dukas, 2010
Fruit fly *Drosophila melanogaster*	Visual learning	Visual. Virgin males trained by mating with a female with a specific eye color for 1.5 h. Number of male courtships and male choice measured in a two-choice assay 24 h after training.	Males preferred females having the same eye color as the one they were trained with.	Verzijden et al., 2015
Fruit fly *Drosophila melanogaster*	Preference for phenotypic novelty	Full phenotype. Training: mating of 5-day-old males with a random female. Male preference and courtship quantity measured in a two-choice assay with live females immediately after training, or with decapitated females 30 min after training (choice between the female the male previously mated with, and a novel female).	Males courted the novel female more. Olfaction was involved as *Orco* mutants didn't discriminate between the two types of females.	Tan et al., 2013
Fruit fly *Drosophila melanogaster*	Auditory plasticity	Acoustic. Females trained with conspecific or heterospecific songs during 6 days from emergence. Male chaining behavior (proxy for sexual arousal) measured 1 day later. Sounds broadcasted with loudspeakers.	Males selectively reduced the response to heterospecific male songs (songs are part of the male courtship ritual) only after having experienced conspecific songs.	Li et al., 2018
Fruit fly *Drosophila melanogaster*	Prior information and social experience	Visual. Training: 6-days-old adult males placed with groups of 6-day-old big (high quality) vs. small (low quality) females for 30 min. Mate choice 1 h after exposure in a two-choice assay.	Males exposed to large females courted large females more often, whereas males exposed and mated to small females courted them more often.	Balaban-Feld and Valone, 2017
Fruit fly *Drosophila melanogaster*	Social experience and courtship learning	Full phenotype. Training: adult *fruitless* knockout males reared in isolation for ∼4 days, then tested for courtship; or housed with conspecific males or females, or with females of other *Drosophila* species for ∼4 days after being reared in isolation for ∼4 days. Male behavior recovery measured (courtship and chaining behavior).	*Fruitless* knockout males (that lost their ability to court) recovered their courtship behavior when housed with groups of males or females. The male's ability to court after training was retained for at least a week after being removed from the group.	Pan and Baker, 2014
Fruit fly *Drosophila melanogaster*	Mate copying	Visual. Training: 3- and 4-days-old males observed a choice and mating of a male with another female of a specific color for 30 min. Mate choice between 2 female colors done just after training, during 30 min.	Male's first courtship was directed more often toward the same female color type they had witnessed mating.	Nöbel et al., 2018a

(Continued)

TABLE 1 | Continued

Common and species names	**Type of learning (as per the authors)**	**Learned signal and methodology**	**Altered behavior**	**References**
Fruit fly *Drosophila melanogaster*	Avoidance learning and mating experience	Full phenotype. Males reared 6–8 days post-eclosion either isolated or with 3 other males, or 3 females; or paired for 24 h at 6 days old with a female (including mating). Presence or absence of male-male courtship in a no choice (2 paired males) and two-choice (between a male and a female) assay at 9-days-old was measured.	Males reared in isolation showed more same sex behaviors than males reared in groups of males or females. Avoidance learning and mating experience impacted the expression of same sex behavior.	Bailey et al., 2013
Bee fly *Megapalpus capensis*	Learning	Visual. Virgin males exposed to deceptive floral forms producing strong mating behavior: Males were released in a pollinator cage with deceptive flowers where their feeding and sexual behaviors when visiting flower forms was recorded for 10 min. Males were also caught from areas where the sexually deceptive flower was absent (inexperienced males) or from areas where it was present experienced males. Males released again in the pollinator cage with deceptive flowers after 10 min. The same behaviors were recorded.	Males showed less mating behavior toward the fly-mimicking spots of the deceptive flower form during their second exposure, or when they were caught in areas with deceptive flowers present.	de Jager and Ellis, 2014
Solitary bee *Eucera berladi* Honeybee *Apis mellifera*	Learning	Visual. Bees exposed to various deceptive or not deceptive flower patterns, and being rewarded with sugar while choosing the right pattern. Landing near a pattern, approaching or turning back from the pattern were recorded while several choices proposed	Labellum patterns of different flowers can be reliably learnt; but patterns of flowers from the same inflorescence cannot be discriminated in a lab set-up. Bees can probably learn to recognize the deceptive flower patterns in the field.	Stejskal et al., 2015
Parasitoid wasp *Lissopimpla excelsa*	Learning	Visual. Wasps landing on a presented deceptive flower and contacting its column were caught, marked and released. Re-capture experiment was done 4 days after, with the same display. Recapture rates within a day and within a week were calculated. Copulation attempts were also recorded with successive wasp visits on the presented flower.	The rate of wasps that visit the deceptive flower decreased highly within a day and a week, but the attempted copulations decreased with successive visits too, suggesting that individuals were learning to recognize the flower.	Weinstein et al., 2016
Sweat bee *Lasioglossum zephyrum*	Habituation	Olfactory. Exposure of males to female odors (impregnated on a filter paper) Subsequent approaches and contact with females and with papers impregnated with females' odors were measured,	Male exposure to specific female odors induces a reduction of sexual contact frequency with females (compared to before exposure). Habituation lasted over 24 h, and was proposed to promote outbreeding and prevent the cost of mating with unreceptive females.	Barrows, 1975
Sweat bee *Lasioglossum zephyrum*	Learning or habituation	Olfactory. Training by housing one adult male with one adult female together, allowing copulation attempts for 10 min First female replaced by a second one whose genetic relationship to the first is known. Number of male copulation attempts for 2.5 min was recorded,	Males rejected females if they were genetically close to the one they were previously exposed to. Males learned to recognize the female odor. The learning promotes outbreeding.	Smith, 1983
Sweat bee *Lasioglossum zephyrum*	Learning	Olfactory. Females sampled from different nests and frozen to create pacifier females. Some females had their odors removed. Number of males approaching and contacting the pacifier females in every different nest for 15 min was measured. Measurements were repeated at every nest, several times.	Males contacted females more during the first than during the second presentation. Nestmates were less attractive than non-nestmates (probably due to them being closely related).	Wcislo, 1987

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TABLE 1 | Continued

Common and species names	Type of learning (as per the authors)	Learned signal and methodology	Altered behavior	References
Parasitoid wasp *Pimpla disparis*	Learning	Acoustic and vibratory. 4- to 7-day-old virgin males released in a patch of non-parasitized hosts and in a patch of near-emergence parasitoids in different locations. Number of males approaching and contacting the hosts was recorded. 24 h later, males released again in patches of non-parasitized hosts, in various locations, and their behavior was recorded again. Vibrations and sounds of the developing parasitoid (14–16 days post-parasitism) when contacted by a male parasitoid were also recorded.	Males revisited the location of hosts containing developing parasitoid in the field and in laboratory experiments. Males stayed with the host when the emergence of the parasitoid (a prospective mate) was imminent. Male contact and proximity with a parasitized pupa induced vibrations of the developing parasitoid, which were used as cues to localize mates.	Danci et al., 2013 Danci et al., 2014
Asian citrus psyllid *Diaphorina citri*	Learning	Olfactory. Training: 1- to 2-week-old adult males mated from a colony of mixed individuals, or mated with a female carrying a food odor for 24 h. Virgin males were from a male colony only. Male attraction to female odor tested in Y olfactometer for 5 min.	Male attraction to female odors significantly increased after a mating experience. Male attraction to the food odor alone did not.	Stockton et al., 2017
African field cricket *Gryllus bimaculatus*	Mating	Full phenotype. Sequential exposure of 6- to 10-day-old post-adult molt males to large and small females (exposure to large, then small, then large and small; or exposure to small then large, then small and large). Male latency to court and eagerness to copulate was measured.	Naive males courted both types of females equally, but mated males courted large females more.	Bateman and Fleming, 2006
Pacific field cricket *Teleogryllus oceanicus*	Juvenile social experience	Acoustic. Juvenile males (stage preceding their penultimate instar) exposed to a mix of six songs. Attraction (time spent near the speaker) of 6- and 13-day-old adult males to the other male's song was measured.	Males reared in silence exhibited more satellite behavior (they spend more time near the speaker) than males reared in a rich acoustic environment. Males from the silent population of Kauai increased their encounter rate with females responding to other callers.	Bailey et al., 2010
Bushcricket *Ephippiger diurnus*	Acoustic experience	Acoustic. Males reared from late instar nymphs in silence, or exposed to songs that had increasing syllable numbers, and to a mix of song made of all the syllable numbers for 6 h a day. At 6–8 days post-adult molt, each male had its number of songs and number of syllable per song recorded.	Male call rates as adults decreased with exposure as juvenile to various male calls compared to males reared in silence. Males invested more in attracting a mate in the absence of competition.	Rebar et al., 2016
Wolf spider *Schizocosa ocreata*	Eavesdropping	Visual and olfactory. Sexually mature adult male (laboratory-reared or field-collected) exposed to video playback of a courting male stimulus for 3 min in combination with female silk cues or with the video only; or males paired in an arena with another courting male for 5 min. Trials videotaped, and focal males scored for frequency of courtship tapping displays (after 3 days of conditioning).	Males copied the courtship dance of other males, from videos or from real males. Field-collected males copied more courtship sequences than lab-reared males. Males also learned to associate the courtship with female cues, as they showed higher levels of courtship when female odors were present. Courtship was also increased when male density was higher.	Clark et al., 2012 Clark et al., 2015
Wolf spider *Hogna helluo*	Exposure	Full phenotype, visual, and olfactory. Exposure without mating: adult females paired with males, no mating allowed, but visual and olfactory cues available; or males kept individually without exposure. Exposure done on days 1 and 3, followed by mating trials on day 4. Exposure with mating: females mated with males, and mating trial done 1 week later. Mating trial: females paired with a male, and mating number, latency and copulation duration were measured.	Males exposed to female cues were less likely to mate with the proposed female compared to control males. Authors propose that exposed males likely perceived a high availability of females and took fewer risks (they became choosier). Previously mated males increased their subsequent mating frequency and had lower risks of cannibalism. These males might be perceived as higher quality mates.	Wilder and Rypstra, 2008

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TABLE 1 | Continued

Common and species names	Type of learning (as per the authors)	Learned signal and methodology	Altered behavior	References
Cellar spider *Pholcus phalangioides*	Experience and learning	Olfactory, tactile and full phenotype. Adult males exposed to the silk of a non-virgin female for 30 min a day over 5 consecutive days; or adult males non-exposed; or, one virgin male paired with one virgin female for 1 h. 24 h after exposure or mating, one male and one non-virgin but sexually receptive female were paired for 30 min. Record of courtship latency, duration, number of copulation attempts and copulation latency.	Males experienced with female silk courted non-virgin females for a longer duration than inexperienced males. Males that were already mated rarely courted a second female and never copulated with the second female in this experiment.	Hoefler et al., 2010
Wolf spider *Schizocosa rovneri*	Experience of female feedback	Visual and seismic. Males trained on a substrate transmitting vibratory cues, or on a hard substrate that didn't transmit the cues. Pre-training evaluated male's baseline substrate use (5 min); followed by a training where males received feedback from a puppet female (they received visual and seismic cues from the puppet, or seismic cues only, or visual cues only). Control males received no feedback. Male subsequent courtship behavior (number and location of body bounce) toward female's odors measured during 5 min right after the training.	Males that experienced feedback from the female puppets increased their number of seismic signals and chose a better substrate for their vibrations. These males that adjusted their courtship were more likely to copulate, reached copulation more quickly, and were less likely to be cannibalized than males that didn't change their signal. This is one of the few studies linking the learning process to fitness effects.	Sullivan-Beckers and Hebets, 2011 Sullivan-Beckers and Hebets, 2014
Burrow-digging wolf spider *Allocosa senex*	Experience rejection by females	Full phenotype. Naïve adult males (with burrows already built) non-exposed or exposed to virgin or mated females. If male was rejected by the female, the male was left for 48 h when he could modify his burrow, and was paired with the same virgin or the same mated female. Burrow dimensions were measured.	Males exposed to females that reject them learn to enlarge their burrows which become longer than unexposed males, and therefore increase their future chances of mating (females prefer large burrows). Here, male learning directly improves his reproductive success.	Carballo et al., 2017
Squinting bush brown butterfly *Bicyclus anynana*	Biased learning	Visual. Newly emerged males exposed to females with either zero or two dorsal hindwing spots for 3 h. 2-day-old male preference measured in a two-choice assay with a zero and two dorsal hindwing spot females.	Males exposed to 0-spot females (on the hindwing) for 3 h learned to prefer them in subsequent mating trials, while naïve males and those exposed to 2-spot females mated randomly.	Westerman et al., 2014
Light brown apple moth *Epiphyas postvittana*	Habituation	Olfactory. Pre-exposure: 1-day-old males exposed to the female's main sex pheromone component. Males' locomotor activity measured before, during, and after the pre-exposure to the component. Males tested for their locomotor activity again 24 h later upon exposure to the female sex pheromone blend.	Males increased their activity during the pre-exposure to the main pheromone component, compared to before and after pre-exposure. The subsequent trial with the complete sex pheromone blend showed lower male activity peak than during the pre-exposure to one pheromone component.	Suckling et al., 2018
Noctuid moth *Copitarsia decolora*	Pre-exposure	Olfactory. 4-day old virgin males exposed to 3 female equivalents of the sex pheromone gland extracts for 10 seconds. Control males were not exposed. Male activation, flying toward the source and landing on the odor source latencies measured 2, 24 and 48 h after exposure in a wind tunnel with the sex pheromone gland extracts as attractor.	Pre-exposure induced an increase in latency of activation and landing at the odor source, so it induced a short-term (2 h) inhibition of the response to the olfactory stimulus.	Robledo et al., 2018
African cotton leafworm moth *Spodoptera littoralis*	Sensitization	Olfactory. 2- to 4-day-old naïve virgin males shortly (~10–30 sec) pre-exposed to 1 female equivalent of sex pheromone extract, or to the main component. Control males were not exposed. From 15 min to 51 h after exposure, male behaviors tested in a wind tunnel where they were recorded for their orientation and movement toward the main pheromone component or gland extracts.	Increased activation and attraction to the odor source in pre-exposed males compared to control males even 51 h after pre-exposure. This behavior is attributed to a sensitization of the primary olfactory centers.	Anderson et al., 2003 Silvegren et al., 2005 Anderson et al., 2007

(Continued)

TABLE 1 | Continued

Common and species names	Type of learning (as per the authors)	Learned signal and methodology	Altered behavior	References
African cotton leafworm moth *Spodoptera littoralis*	Sensitization	Acoustic. Pre-exposure of naïve males to pulsed bat-like sounds (predator sounds). Control males were not exposed. Male behavior tested in a wind tunnel where mare orientation and movement toward the sex pheromones were recorded.	Increased behavioral and neuronal sensitivity of the male to female sex-pheromones upon exposure to predator sounds. Moths were thus capable of integrating bimodal sensory information.	Anton et al., 2011
African cotton leafworm moth *Spodoptera littoralis*	Experience	Olfactory. Laboratory set-up: males reared as larvae on cotton leaves or cotton leaves or artificial diet until pupation followed by measurement of 3–4-day-old male attraction to female sex pheromones associated with plant odors in a two-choice wind tunnel assay in the laboratory. Field work: larvae reared on cotton or on alfalfa, then 8-day-old pupae were transferred to the border between cotton and alfalfa fields. Pheromone traps baited with the major component of the female sex pheromone blend were placed in the fields. Number of males captured in each field was recorded over 10 days.	Males were more attracted to female sex pheromones combined with the odor of the host plant species they experienced as larvae than to sex pheromones combined with odor from the plant they had not experienced. By selecting a female on a plant of the same species that supported his own larval development, the male controls the plant quality for his offspring.	Anderson et al., 2013
Rove beetle *Aleochara curtula*	Habituation and learning	Full phenotype, olfactory, gustatory and tactile. Males presented with a mated female pacifier five times in succession. Some female pacifier cuticular hydrocarbons were manipulated to make them either similar or different in scent and presented one after the other. First presentations were followed by five additional presentations of either another mated or a virgin female pacifier. Males grasping responses on females and pacifier were measured at each presentation.	Males learned to recognize the cuticular hydrocarbons of the females they mated with and reduced subsequent mounting attempts with these females.	Schlechter-Helas et al., 2012
Familiar bluet damselfly *Enallagma civile*	Learned mate recognition	Visual. There are differently colored female morphs in the species. Sexually mature males caught in the field, marked, and released inside an enclosure with equal number of andromorphic or heteromorphic females. Interactions happened for 2 days. Males presented to live tethered females of different morphs, at different times of the day, and male behavior was recorded: no reaction, approach, grab and tandem formation (copulation). Males were also tested in a two-choice assay with females of different morphs.	Sexually mature males learned to prefer the color morph (including the andromorphic females) they have been previously exposed to (interacting and mating). Naïve males didn't have a preference. They learned the most common morph encountered in the field.	Miller and Fincke, 1999 Miller and Fincke, 2004 Fincke et al., 2007
Familiar bluet damselflies *Enallagma aspercum* *E. civile*	Experience	Full phenotype and visual. Female morph frequencies were tallied during the morning and the afternoon in both species. Male sexual behavior (approach, grab, tandem or takeover of another mating) measured when presented to tethered females of the different species in the morning or in the afternoon, when frequencies of each morph were different.	Males of both species reduced their heterospecific sexual interactions with females in the afternoon, after having interacted with them. The male's sexual response toward female morphs of both con- and heterospecifics varied over the course of a day in response to changes in the density of female morphs.	Miller and Fincke, 2004
Common bluetailed damselfly *Ischnura elegans*	Habituation	Visual. There are differently colored female morphs in the species. Successive exposure of males to different female morphs or to other males during 2 days. Measure of male preference after each exposure by two-choice assay with two female morphs proposed to the male for mating.	Males preferred the most recently encountered female morph. Males changed their preference after each successive encounter based on the most common morph. Male chose their mate in a frequency dependent way. Males exposed to males also formed a majority of tandems (copulation attempts) with other males, but when these males were housed again with a mix of males and females, they preferred the more abundant female morph.	van Gossum et al., 2001 van Gossum et al., 2005

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TABLE 1 | Continued

Common and species names	Type of learning (as per the authors)	Learned signal and methodology	Altered behavior	References
Marsh bluetailed damselfly *Ischnura senegalensis*	Experience and copulation	Visual. Lab assay: 5- to 9-day-old sexually mature virgin adult males paired with a single sexually mature female, either an andromorph or a gynomorph, in the morning for 4 h. Control males were reared isolated. Fieldwork: Female morph frequency tallied in the field, in the morning and in the afternoon. Male preference tested in a two-choice assay with both female morphs (female dummies) in the afternoon in the lab, and in the morning and afternoon in the field.	Virgin males had no innate mating preference for a female morph, but males that experienced copulation significantly preferred the same morph they copulated with. In the morning, males didn't discriminate between female morphs, but they preferred the dominant female morph in the afternoon. Males changed their preference based on the most common morph present in the field.	Takahashi and Watanabe, 2010a Takahashi and Watanabe, 2010b
Marsh bluetailed damselfly *Ischnura senegalensis*	Learning	Visual learning. Virgin male paired with a sexually mature female gynomorph or andromorph in the morning until mating. Control males were reared isolated. Immature and mature gynomorphs have brightness differences not shown by andromorphs. 6–9 day old sexually mature males submitted to two-choice assays with immobilized sexually immature (1- to 3-day-old) and sexually mature females (5- to 9-day-old) of both morphs.	Virgin males didn't have a preference between an immature and mature female, but males that had experienced copulation with gynomorphs preferred sexually mature gynomorphs to sexually immature ones. Males experienced with andromorphs didn't discriminate. Males might be learning the color differences between mature and immature gynomorphs.	Takahashi and Watanabe, 2011
Treehopper *Enchenopa binotata* species complex	Social experience	Acoustic. Adult males exposed 1 h per day for 14 days to a range of male calling frequencies corresponding to con- and heterospecific signals, to a mix of signal frequencies, or to silence. Males also exposed to a mix of male calls and to different female signal responses. Male's signals the day after their last exposure (minimum 18 h) were recorded.	Males exposed to a range of conspecific competitors (mimicked by signals with average frequencies) signaled faster (higher rate) than non-exposed males or males exposed to unattractive signals. Also, males called for longer time when they were exposed to females that responded to their preferred male call frequency.	Rebar and Rodriguez, 2016

a male. Females in many species also prefer a new male over their previous mate. For instance, in crickets (Bateman, 1998), in moths (Xu and Wang, 2009; Li et al., 2014), in hide beetles (Archer and Elgar, 1999), or in female *Drosophila melanogaster*, a simple exposure without mating is enough to trigger a similar preference for a new male (Odeen and Moray, 2008; Loyau et al., 2012). This type of learning might also be adaptive as by rejecting the males that they saw copulating, females could reduce the costs of mating with semen-limited males (Loyau et al., 2012).

Visual Signals

Multiple studies have shown that naïve individuals have no innate genetic mate preferences for particular visual signals but develop these through learning. In many cases, naïve males direct their courtship toward a wide range of females, while inexperienced females display no preference for a specific male visual trait. For instance, male fruit flies, who initially court both large and small females with equal vigor, will preferentially court one of these female types if previously exposed or mated to them (Balaban-Feld and Valone, 2017). In wolf spiders, juvenile or adult exposure to male tibia types is necessary to limit the female preference to a specific leg tuft size or color (Hebets, 2003, 2007; Rutledge et al., 2010; Stoffer and Uetz, 2015, 2016a,b). Male damselflies learn to prefer the female color morphs they previously interacted with (Miller and Fincke, 1999, 2004; van Gossum et al., 2001; Fincke et al., 2007; Takahashi and Watanabe, 2010b, 2011). Experienced female damselflies learn to reject heterospecific males by recognizing their wing patches (Svensson et al., 2010, 2014; Verzijden and Svensson, 2016). The acquisition of a preference for visual traits has also been reported in fruit flies [eye color, (Verzijden et al., 2015)], butterflies [hindwing ornamentation number, (Westerman et al., 2014)], or crickets [size, (Bateman et al., 2001)] (**Figure 2**). Recent studies on mate-choice copying showed that virgin individuals tend to prefer male phenotypes with similar color type and ornamentation as the mate choice of another conspecific, e.g., in spiders, (Fowler-Finn et al., 2015) and fruit flies, (Mery et al., 2009; Nöbel et al., 2018a). These cases illustrate that insects can generalize socially learned public information for choosing a mate. Finally, male spiders can also copy the leg-tapping dance of other courting males to increase their likelihood of seducing a female (Clark et al., 2012, 2015; **Figure 2**). These males reach copulation more quickly and are less likely to be cannibalized (Sullivan-Beckers and Hebets, 2011, 2014).

In the few documented cases of insects displaying innate (naïve) visual signal preferences, learning can be context dependent, and sometimes may override, or even reverse these preferences. For instance, female *Bicyclus anynana* butterflies can switch their naive preference from males with two UV-reflective spots on their forewings to four spots if they are

TABLE 2 | A non-exhaustive list of publications about how female insects and spiders learn sexual behaviors.

Common and species names	Type of learning (as per the authors)	Learned signal and methodology	Altered behavior	References
Fruit fly *Drosophila melanogaster*	Mate copying	Visual. Training: 3-day-old female observations of a mating pair with male of a certain color and a virgin female (30 or 60 min), followed by the rejection of a male of another color by an already mated female (1 h). Sequence repeated 3 times; or short demonstration of 30 min only. Measure of female mate choice in a two-choice assay.	Females preferred to mate with the same male color type they previously observed mating. Naïve females had no preference. Mate copying is stronger when the demonstration happened sequentially, and in higher atmospheric pressure (better weather conditions).	Mery et al., 2009 Germain et al., 2016 Dagaeff et al., 2016 Nöbel et al., 2018b
Fruit fly *Drosophila melanogaster*	Mate copying avoidance	Visual. Training: 3-day-old female observations of both a mating pair and of a male being rejected by a female for 45 min. Measure of female mate choice in a two-choice assay just after the observation.	Females preferred to avoid the specific male they saw copulating before and mated with the other male. By rejecting the males they saw copulating, females could reduce the costs of mating with semen-limited males.	Loyau et al., 2012
Fruit fly *Drosophila melanogaster*	Exposure	Full phenotype. Training: 1-day-old virgin female housed but separated from 2 males with netting for 8 h. Mating trials started 12 h later, with the same males, or a novel male, until copulation.	Females preferred to mate with a non-familiar male than with the ones they were housed with, even if they didn't copulate with the earlier male.	Odeen and Moray, 2008
Fruit fly *Drosophila melanogaster*	Female's social experience	Full phenotype. Training: <1-day-old adult females of various genetic backgrounds housed with high-harm males (negatively impacting females' fecundity) and low-harm males (low effect on females' fecundity) for 3 h (+45 h separated from males) or 48 h. Female mate choice between a high- and a low-harm male recorded right after the 48 h.	Females housed and mated with low-harm males spent more time subsequently interacting with any male, compared to females that mated with high-harm male genotypes. Here, the behavioral plasticity in female mate choice behavior is mediated by indirect genetic effects associated with their former mating experience.	Filice and Long, 2017
Fruit fly *Drosophila melanogaster*	Auditory plasticity	Acoustic. Females trained under conspecific or heterospecific songs during 6 days from emergence. Female latency to copulate with one male measured 1 day later. Sounds broadcasted with loudspeakers.	Training with conspecific male songs reduced female acceptance of heterospecific songs (copulation acceptance).	Li et al., 2018
African field cricket *Gryllus bimaculatus*	Exposure	Full phenotype and visual. Adult female sequential exposure and mating to males of different sizes (every ∼30 min). Female acceptance or rejection, mating latency and spermatophore retention recorded for each male presentation.	Females previously mated with males of any size favored big males in subsequent matings, compared to virgin females who didn't discriminate between male sizes. This process may help a female ensure that she is choosing the most attractive mate available in her social environment.	Bateman et al., 2001
Hawaian cricket *Laupala cerasina*	Exposure	Acoustic. More than 14-day-old virgin adult females sequentially exposed to songs, twice per day for 3 days. Female attraction to the speaker was measured.	Female responsiveness (attraction to a preferred song) decreased with exposure to a series of songs.	Shaw and Herlihy, 2000
Pacific field cricket *Teleogryllus oceanicus*	Social experience	Acoustic. 5- to 8-day-old virgin females exposed to preferred and non-preferred male courtship songs during mating trials with silenced males (< than 10 min). 24 h later, female latency to accept copulation and latency to reject spermatophore after copulation were measured.	Females mated with males with preferred (= attractive) songs mounted subsequent males more slowly and had lower subsequent sperm retention durations than females mated with males with non-preferred songs.	Rebar et al., 2011
Variable field cricket *Gryllus lineaticeps*	Acoustic experience	Acoustic. 7- to 30-day-old virgin females exposed to sequences of songs with different chirp rates, naive females preferring high chirp rate songs. Female distance to the speaker was measured as a song attractiveness proxy in three sequential trials separated by a 20 min resting period.	Females exposed to a sequence of attractive (high chirp rate) and unattractive songs were more attracted to attractive songs than females exposed to unattractive songs only.	Wagner et al., 2001
House cricket *Acheta domesticus*	Exposure to conspecifics	Full phenotype. Adult females reared in isolation or in low and high density groups (with equal numbers of males and females). Courtship trials conducted when females were ∼10- and ∼32-day-old. Frequency of female approaching males and number of mounts were measured.	Females reared in groups approached and contacted males more frequently than females reared in isolation.	Tinghitella, 2014

(Continued)

TABLE 2 | Continued

Common and species names	Type of learning (as per the authors)	Learned signal and methodology	Altered behavior	References
Black field cricket *Teleogryllus commodus*	Effect of social environment	Acoustic. Female nymphs within 24 h of hatching exposed to different song intercall durations, mimicking different male densities and calling rates. Upon maturity, females isolated for 10 days. Then, female choice measured in a two-choice assay, each female used in 6 consecutive choice trials.	Females reared in a "middle calling song rate" acoustic environment decrease their response latency toward future calls, compared to females reared in low or high calling rate acoustic environments. Females responded more quickly in later trials.	Kasumovic et al., 2012
Pacific field cricket *Teleogryllus oceanicus*	Social learning	Acoustic. 5 min exposure of 6- to 7-day-old females to different calling song models (preferred and non-preferred by the females). Female subsequent attraction to the speaker playing a reference song measured after 30 s rest.	Females that experienced preferred songs had lower attraction to the reference song compared to females exposed to other songs. Females experienced with less-preferred songs showed higher attraction to the reference song.	Bailey and Zuk, 2009
Pacific and black field crickets *T. oceanicus* *T. commodus*	Social learning	Acoustic. Juvenile females from 4th instar reared in silence or exposed to a mix of male calling songs that mimicked a natural setting. 6-day-old mated females also exposed to songs and silence as adults. Female phonotaxis - attraction to the speaker playing different calling songs - tested 6–10 days post eclosion, each with 16–24 h of silence prior to testing. Female phonotaxis also tested in a two-choice assay between con- or heterospecific songs.	Adult or juvenile females reared in silence (mimicking the Hawaiian Kauai silent population) were more responsive (more attracted) to future callings than females reared in a mix of songs. Females seem to accommodate the loss of sexual signal in this population.	Bailey and Zuk, 2008 Bailey and Macleod, 2014 Swanger and Zuk, 2015
Pacific field cricket *Teleogryllus oceanicus*	Social flexibility	Acoustic and indirect genetic effects on female preferences. Females from 5 different populations exposed to silence or to a mix of male calling songs that mimicked a wild setting with a high density of calling males. 6–10 days post eclosion female placed with one silent male to estimate females' latency (=choosiness) to mount.	Female's previous experience altered their choosiness depending on studied populations. In Hilo populations, female choosiness decreased with song experience, while in the Samoa and Oahu populations, experience didn't affect female behavior, and contemporary Kauai females (from a silent population) were choosier after exposure to male songs, whereas ancestral Kauai females were less choosy after exposure to male songs. Indirect genetic effects affect mate choice and are likely to have an evolutionary impact of the populations.	Bailey and Zuk, 2012
Bushcricket *Ephippiger diurnus*	Experience, rearing environment	Acoustic. Rearing of females from late instar nymphs to 13-day post- adult molt in silence, or exposed to songs that have increasing syllable numbers, and to a mix of songs made of all the syllable numbers for 6 h a day. After 24 h, each female tested with several acoustic stimuli from 1 to 10 syllables. Females' movements toward the sound source recorded with a locomotion compensator sphere. Construction of the female mate preference function to measure her selectivity (see **Figure 1**).	Females from every acoustic environment preferred songs with high syllable rate. The females exposed to a mix of songs were more selective than the other females.	Rebar et al., 2016
African field cricket *Gryllus bimaculatus*	Mating	Full phenotype. Pairing of adult virgin males and females until copulation. 12 h later, mate choice between the same male and a different male was recorded.	Females preferred to re-mate with a novel male rather than with a male they already mated with. They gain benefits from multiple mating.	Bateman, 1998
Wolf spider *Schizocosa uetzi*	Exposure and subadult experience	Visual. Subadult females (penultimate stage before molting into adult) exposed to courting males with the tibia and patella painted in brown or in black for 30 min per day until final molt, control were non-exposed females. 11- to 20-day-old females paired with a male of one of the two phenotypes, and occurrence of copulation, latency to copulation, and occurrence of cannibalism, during 30 min trials was recorded.	Exposed females were more likely mate with a male having the same color as the one they were exposed to. Females were also more likely to cannibalize a male with non-familiar color. Control naïve females had no bias for either morph.	Hebets, 2003

(Continued)

TABLE 2 | Continued

Common and species names	Type of learning (as per the authors)	Learned signal and methodology	Altered behavior	References
Wolf spider *Schizocosa rovneri*	Exposure and subadult experience	Visual. Subadult (penultimate instar) females exposed to courting adult conspecific males with forelegs painted with black or transparent nail polish, or with a black or transparent nail polish dot on the cephalothorax (odor-control males). 7–21 days following adult molt, female receptivity or willingness to mate with males of the different phenotypes was measured.	Females exposed to black foreleg males were later less receptive to these specific males, and less likely to mate with them, than to the males painted with transparent nail polish, and to control males. Females exposed to legs painted with transparent nail polish preferred males with black paint over other males. Unexposed females showed no innate preference. Difference between these results and Hebet's earlier work (2003–above) might be explained by differences in experimental set-up (e.g., the type of paint used, as the paint odor might impact the female's response toward the visual cue).	Rutledge et al., 2010
Wolf spiders *Schizocosa ocreata* *S. rovneri* (sympatric species, reproductively isolated by courtship alone)	Exposure and subadult experience	Visual. Subadult females (last instar before adult molt) exposed to courting brushlegged males (*sensu S. ocreata*) or non-ornamented males (*sensu S. rovneri*) every 2–3 days during 30 min each. Non exposed females are control. Female paired with one of the male forms 13–24 days after their adult molt. Occurrence of copulation, latency to copulation, and occurrence of cannibalism, during 30 min trials was recorded.	Experienced females preferred brushlegged males (*S. ocreata*), regardless of the male they were exposed to, whereas inexperienced females showed no mating preference (between ornamented and non-ornamented males *sensu S. rovneri*).	Hebets and Vink, 2007
Wolf spiders *Schizocosa ocreata* *S. rovneri* (allopatric species)	Prior juvenile experience	Visual and seismic. Subadult females (last instar before adult molt) exposed to conspecific or heterospecific courting males every day during 30 min each. Control females were not exposed. Contact between individuals not allowed (they shared visual and seismic cues only). Females paired with one of the males 7–14 days after their adult molt. Female receptivity behavior and occurrence of female aggression or cannibalism was recorded.	No effect of pre-exposure: exposed or unexposed females preferred their respective male species. More exposure to conspecifics reduced females' *S. ocreata* aggressiveness toward conspecifics. Exposure to heterospecific male courtship did not affect aggression in *S. ocreata*. Exposure didn't affect *S. rovneri* aggressiveness.	Rutledge and Uetz, 2014
Wolf spider *Schizocosa ocreata*	Socially cued anticipatory plasticity	Visual. Subadult females (last instar before adult molt) exposed to videos of courting males with small or average, or large tibia tufts (foreleg bristles) size, or a mixture of sizes, until adult molt. Exposure done at various frequencies. Female receptivity behavior for courting males (on videos) tested at 10–15 days after their adult molt in no-choice (males with large or small tufts) and two-choice assays (large and small tufts).	Females exposed to average leg tuft size, a mix of sizes, and large tufts were more receptive to future courtship of large-tuft males over small-tuft males. Females exposed to small-tuft males were later more receptive to future courtship of small-tuft males than large-tuft males (when exposed as juvenile or as adults). Naïves didn't have a preference.	Stoffer and Uetz, 2015 Stoffer and Uetz, 2016a
Wolf spider *Schizocosa ocreata*	Adult social experience	Visual. to-6-days-old adult females exposed to videos of courting of males with small or large tibia tufts. Frequency and number of exposure varied. Female receptivity behavior toward video of 7-day-old courting males in a two-choice assay (large and small tufts).	Females pre exposed to small-tuft males displayed more receptivity toward small-tuft males, while those exposed to large-tuft males displayed more receptivity toward large-tuft males.	Stoffer and Uetz, 2016b
Wolf spider *Schizocosa ocreata*	Courtship modality experience	Visual and seismic. Subadult females (last instar before adult molt) exposed to videos of courting males with an average leg tuft size, or exposed to a vibratory playback (mimicked courtship seismic signal), or exposed to both. Control females were not exposed to any signal. Exposure ended at the final molt to adult stage. Female's latency to approach the source of each signal, and of the receptivity behaviors at 10–15 days after their adult molt was measured in no-choice (one female was tested with the visual signal only, then the seismic signal only and then both signals) and two-choice assays (visual and seismic).	Female juvenile experience didn't affect their subsequent latency to approach the signal, as they all approached the courtship signals faster when playback included visual signals (visual only and multimodal). Females displayed more receptivity toward the unimodal signal modality they were exposed to, but they preferred the multimodal signal above all other ones, regardless of exposure (in the no choice assay).	Stoffer and Uetz, 2017

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TABLE 2 | Continued

Common and species names	Type of learning (as per the authors)	Learned signal and methodology	Altered behavior	References
Wolf spiders *S. ocreata* *S. rovneri*	Mate choice copying and learning	Visual and seismic. Virgin females exposed visually to another female choosing between a male with a tibia tuft and a non-ornamented male. The female was exposed to the courtship and copulation happening to the other female or to the copulation only. Control females were not exposed. The exposed female could perceive the visual and vibratory signals of the interacting trio, but could not physically interact with them. Female preference for the ornamented or non-ornamented male tested in a two-choice assay just after the exposure.	Exposed females matched the mate choice (ornamented vs. non-ornamented males) of the females they observed being courted and mated.	Fowler-Finn et al., 2015
Wolf spider *Hogna helluo*	Exposure	Full phenotype, visual, and olfactory. Exposure without mating: adult females paired with males, no mating allowed, but visual and olfactory cues available; or females kept individually without exposure. Exposure done on days 1 and 3, followed by mating trials on day 4. Exposure with mating: females mated with males, and mating trial done 1 week later. Mating trial: females paired with a male, and mating latency, copulation duration and female aggression frequency were measured.	Mated females had lower probability to mate than virgin females. Females exposed to visual and olfactory cues took less time to mate than non-exposed females. Previously mated females were significantly more likely to engage in precopulatory cannibalism than virgin females.	Wilder and Rypstra, 2008
Fishing spider *Dolomedes triton*	Exposure	Full phenotype. Juvenile female (∼10 days before adulthood) housed with a mature male, a juvenile female, or kept isolated. 4 days after adulthood, females were paired with an adult male for 1 h or until mating happened. Occurrence of female precopulatory attacks recorded.	Virgin females exposed to males as juveniles showed more pre-copulatory attacks than females exposed to another female, or than non-exposed females. Whatever the exposure treatment, virgin females were more likely to attack males than already mated females.	Johnson, 2005
Squinting bush brown butterfly *Bicyclus anynana*	Biased learning	Visual. Females exposed on the morning of their emergence for 3 h to a single virgin male with zero, 2 or 4 forewing dorsal spots. Naive females were not exposed and kept isolated until mate choice trial. Some males used for exposure had their sex pheromone producing organs blocked. 2-day-old female preference measured in a two-choice assay, between a zero and a 2-spot male, or between a 2 and a 4-spot male.	Females switch their innate preference from 2-spot males (on the forewing) to 4-spot males if exposed to them upon emergence. Females don't learn to prefer 0-spot males. When sex pheromones are blocked, females learn to avoid the 4-spot males.	Westerman et al., 2012 Westerman and Monteiro, 2013
Squinting bush brown butterfly *Bicyclus anynana*	Learning	Olfactory. Females exposed on the morning of emergence to males with a wild-type sex pheromone blend, or a reduced sex pheromone blend (lacking one component and the two other highly reduced in quantity), or an enhanced blend (one component increased by perfuming). 2-day-old female preference measured in a two-choice assay with the wild-type and the reduced blend males, or with the wild-type and the enhanced blend males. Preference of 2-day-old naive female offspring of the females exposed to reduced blend, and of offspring of females exposed to wild-type blends measured in a two-choice assay with a reduced blend male and a wild-type blend male.	Females exposed to wild type blends find the reduced blend unattractive, but when exposed to the reduced blend, find this blend as attractive as the wild type blend. Females exposed to the enhanced blend learn to prefer it over the wild type blend. Offspring of females exposed to the reduced blend stop showing a preference for the wild type blend, compared to offspring of females exposed to wild type blends. This is the first study that shows inheritance of a learned response to a pheromone blend.	Dion et al., 2017
African cotton leafworm moth *Spodoptera littoralis*	Exposure	Olfactory. Females exposed to a 0.1 female equivalent of the sex pheromone extract for 4 min, within 2 min from the beginning of scotophase or 3 h before the onset of the expected scotophase on the first 2 days after emergence. Control females were exposed with the solvent used for pheromone extraction. Female calling behavior measured in a wind tunnel during the 4 first days after emergence.	Exposed females increased the proportion of subsequent calling and called longer than non-exposed females. The effect was persistent for at least two additional days. This behavior could be advantageous under high population densities with high competition between females.	Sadek et al., 2012

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TABLE 2 | Continued

Common and species names	**Type of learning (as per the authors)**	**Learned signal and methodology**	**Altered behavior**	**References**
African cotton leafworm moth *Spodoptera littoralis*	Mating experience	Full phenotype. 1-day-old male and female virgin moths paired for mating. 24 h after mating, the female was repaired with her previous mate, or with a novel male and her calling behavior was evaluated.	Previously mated females called earlier and longer when paired with a novel male than when paired with their previous mate.	Li et al., 2014
Mediterranean flour moth *Ephestia kuehniella*	Mating experience	Full phenotype. 1-day-old male and female virgin moths paired for mating. 14 h later, female choice measured in a two-choice assay with previous and new male, or in a no-choice assay with a new or the previous male she copulated with.	Females chose preferentially a new male for their second mating.	Xu and Wang, 2009
European grapevine moth *Lobesia botrana*	Exposure	Olfactory and full phenotype. 1-day-old female exposed housed with four 1-day-old females (calling, emitting sex pheromones) or housed alone. Female calling behavior recorded every night (for 5 nights) every 10 min for 130 min.	On the first night, exposed females signaled at a higher rate than females kept alone. The following nights, exposed females gradually signaled less than isolated females.	Harari et al., 2011
Hide beetle *Dermestes maculatus*	Mating experience	Full phenotype. 1– to 4-weekold females paired with males until copulation. 20 min after first copulation, females were paired with the same male or a novel male.	Females presented with a new male mated faster and rejected males less than when presented with their previous mate.	Archer and Elgar, 1999
Banded demoiselle damselflies *Calopteryx splendens* *C. virgo*	Learning	Full phenotype and visual. Males from the two different species are recognized thanks to their different wing patches. Sexually experienced females were caught in the field, or they were housed with each male morph for 2 h. Control females were reared isolated in the lab. Individuals from both species came from sympatric or allopatric populations. Female mate choice tested in a two-choice assay with tethered *C. virgo* and *C. splendens* males, before and after the social experience.	Virgin *C. splendens* females did not discriminate against heterospecific males based on wing patches, but experience, including mating, made them learn to reject heterospecific males. This learning helps species recognition in sympatric populations, and promotes reproductive isolation.	Svensson et al., 2010 Svensson et al., 2014
Banded demoiselle damselfly *Calopteryx splendens*	Learning	Visual. *C. virgo* male wing patch is fully melanized while only partially melanized in *C. splendens*. Experienced females were caught in the field, or exposed to a locally caught *C. splendens* male for 1 h followed by exposure to a *C. virgo* male, or vice versa. Control females were reared in isolation. Female preferences for male wing patch size tested by presenting conspecific tethered *C. splendens* males with manipulated wing patches varying in size.	Field experienced *C. splendens* females from sympatric populations with *C. virgo* preferred small male wing patches, whereas females from the allopatric population preferred large patches. Females from the sympatric population exposed to a conspecific male developed preference for smaller wing patch sizes, whereas females from the allopatric population exposed in the same way didn't develop such a preference. Control females didn't have a preference. Co-occurrence with a closely related species caused *C. splendens* females to prefer male traits that are more species specific.	Verzijden and Svensson, 2016
Treehopper *Enchenopa binotata* species complex	Social plasticity	Acoustic. 2- to 3-week-old virgin females (before their sexual receptivity) exposed for ∼2.5 weeks (50 min per day) to the preferred (the population peak preference) male call frequency, or to lower or higher frequencies (non-preferred, overlapping with heterospecific male calls), or to a mixture of call frequencies, or to silence. 4- to 5-week-old female response signal measured when tested with various male call frequencies. The female preference curve was built to measure her selectivity and determine changes in peak preference (**Figure 1**).	Females preferred the mean call frequency of the males from the same population (this mean is the female peak preference). Females exposed to the preferred call frequency (their population peak preference) and females exposed to a mix of preferred and non-preferred call frequencies, increased their subsequent selectivity (lower responsiveness and tolerance and higher strength of preference) for the preferred call. Females exposed to silence, or non-preferred call frequency, didn't change their selectivity.	Fowler-Finn and Rodríguez, 2012a Fowler-Finn and Rodríguez, 2012b

(Continued)

TABLE 2 | Continued

Common and species names	Type of learning (as per the authors)	Learned signal and methodology	Altered behavior	References
Treehopper *Enchenopa binotata* species complex	Social plasticity	Acoustic. Females reared in populations with controlled genetic background (full sib families), or in populations with different male calling rates, or in populations with different densities. After reaching adulthood, females were kept isolated. Sexually receptive female (∼7-weeks-old) response signal measured when tested with various male call frequencies. The female preference curve was built to measure her selectivity and determine changes in peak preferences (see **Figure 1**).	There was significant genetic variation in social background on peak preference and on female selectivity. Female preferences also varied with local density, with higher signal frequencies being preferred in denser environments.	Rebar and Rodríguez, 2013 Fowler-Finn et al., 2017

exposed to these males shortly during sexual maturation Westerman et al. (2012). Importantly, learning is context dependent, as female butterflies learn to avoid, rather than prefer, the novel wing patterns when the male sex pheromone is absent in the training male (Westerman and Monteiro, 2013). This suggests that olfactory communication may trump visual communication in assessing mates, at least in *B. anynana* butterflies. In addition, learning can be biased in that some sexual signals (supernumerous eyespots) can induce an increased preference while exposure to others (fewer eyespots) does not modify innate mate preferences (Westerman et al., 2012).

Olfactory Signals

In multiple studies, the learned sexual signal that triggers a behavioral change is an olfactory signal. Innate preferences appear to be generally present for olfactory signals in insects, and they can trigger a wide range of behaviors in receivers of many species from the same or the opposite gender. Odor learning can lead to either habituation or to sensitization, which led to opposite behavioral responses, but it is still unclear how the two processes work. Already ∼35 years ago, studies reported how male sweat bees avoided mating with previous mates, or with females genetically close to their first mate, by learning to recognize their particular odor (Barrows, 1975; Smith, 1983; Wcislo, 1987). Similar processes were recently reported in rove beetles and fruit flies (Schlechter-Helas et al., 2012; Tan et al., 2013). Such "habituation" to female odor also occurs in male moths, which reduce their level of response to female sex pheromones shortly after exposure to components of the blend (e.g., Robledo et al., 2018; Suckling et al., 2018). On the contrary, increased behavioral sensitivity to sex pheromones, termed "sensitization," was also described in *Spodoptera littoralis* moths whereby sexually mature adult males increase their responsiveness and attraction to the odor source 24 h after exposure (Anderson et al., 2003, 2007; Silvegren et al., 2005). Male *S. littoralis* also learned to prefer a mating site based on a learned plant odor, and reproduced preferentially with females found on the same plant where they grew up as larvae, or on plants where they previously mated (Anderson et al., 2013; Thöming et al., 2013; Proffit et al., 2015). Female *B. anynana* butterflies not only become sensitized to wild-type male sex pheromone composition, but can also learn to prefer unattractive blends if exposed to these blends during sexual maturation, right after adult emergence (Dion et al., 2017; **Figure 2**). Female moths also perceive the sex pheromone of other females, which induce them to emit their own sex pheromone earlier and at higher amount than inexperienced individuals (Stelinski et al., 2006; Sadek et al., 2012). Finally, the presence of antiaphrodisiacs, transferred by males onto the female cuticle, and reproductive tract led other males to learn to avoid mated *Drosophila* females (Ejima et al., 2005, 2007).

Acoustic Signals

Multiple studies have shown a change in sexual behavior upon exposure to acoustic signals, which are often used in species recognition and mate quality assessment in insects (Hedwig, 2016). For example, the rate and number of male calls that female crickets hear as juveniles or during mating significantly affects their preference and their response speed to future mate calls (Wagner et al., 2001; Rebar et al., 2011; Kasumovic et al., 2012). Contrary to individuals exposed to a mixture of call frequencies, females reared in silence respond faster toward a model song mimicking the populations' average calling rate (Bailey and Zuk, 2008, 2009; Bailey and Macleod, 2014; Swanger and Zuk, 2015). Males reared in silence intercept more females attracted to other males' calls and increase their own call rates (Bailey et al., 2010). Changes in a females' response to acoustic experience are variable and population-specific (Bailey and Zuk, 2012). The acoustic environment also impacts female treehopper's preference selectivity (**Figure 1C**) for male signal frequency and speed (Fowler-Finn and Rodríguez, 2012a,b; Rebar and Rodriguez, 2016; Fowler-Finn et al., 2017). In addition, naïve female *Drosophila* initially show no preference to the courtship songs (wing vibrations) of conspecific or heterospecific males, but a pre-exposure to conspecific songs makes them prefer this song type (Li et al., 2018). In some parasitoid wasp species, males identify host pupae parasitized by a conspecific using acoustic and vibratory signals, learn their location, and visit them regularly, as a strategy to attain prospective emerging female mates (Danci et al., 2013, 2014). This is one of the few cases where the adaptive value of learning is highlighted.

TABLE 3 | Summary of the different types of learning in sexual selection in insects and spiders. Modified from (Yeh and Servedio, 2015) and (Varela et al., 2018).

Type of learning	Terms used in the insect learning literature	Sex that learns	Demonstrator	Trait learned from the demonstrator (preference or signal)	Outcome of learning (change in preference or signal)	Outcome of learning producing either a positive or a negative expression of preference or of signal
Sexual imprinting (oblique or horizontal) Based on observing surrounding individuals. Occurs before sexual maturity. Expected to have lifelong effects, although this is rarely tested.	Early exposure, early experience	Male or female	Conspecific or heterospecific	Signal	Preference or signal	Positive toward conspecifics, can be negative against heterospecifics
Imitation Based on observing mating of other individuals. Occurs in sexually mature adults The durability of this mechanism of learning is unknown.	Mate choice copying	Male or female	Conspecific	Preference or signal	Preference	Usually positive, but can be negative if learning occurs by observing failed mating attempts
	Signal copying, eavesdropping	Male		Preference or signal	Signal	
Previous personal experience. Based on interactions with potential and previous mating partners. Occurs in sexually mature adults. The durability of this mechanism of learning is unknown.	Changing preference based on social feedback	Male or female	Conspecific or heterospecific	Preference	Preference	Can be both
	Discrimination based on prior exposure	Female		Signal		
	Signal learning	Male		Preference	Signal	
	Signal learning from social feedback on own attractiveness			Signal		

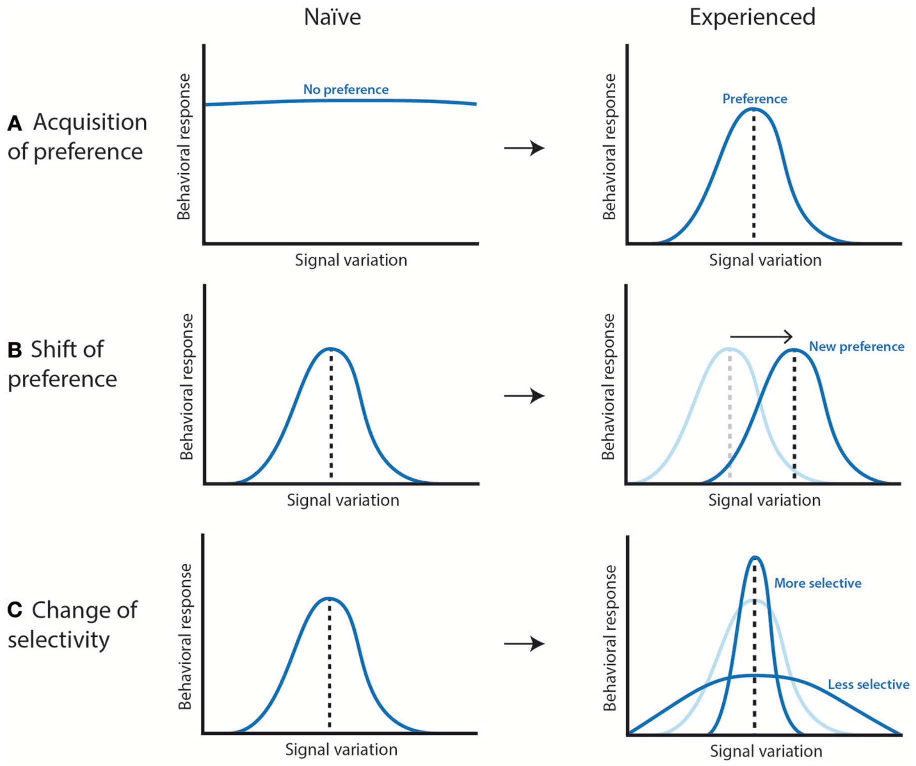


FIGURE 1 | Illustration of how learning can affect a mate preference function. All graphs describe the mate preference of an individual to increasing amount of a sexual signal. **(A)** Individuals "acquire a preference" when they do not have an innate preference, but acquire one through experience. **(B)** Individuals "shift their preference" if they have an innate preference for one signal which is changed to another one after experiencing it. **(C)** A "change of selectivity" involves other types of modifications of the mate preference function, which depends on the individual responsiveness, tolerance and strength of response to a continuously distributed trait. Details on the measure of selectivity can be found in Fowler-Finn and Rodríguez (2012a,b) [Figure adapted from Fowler-Finn and Rodríguez (2012b)].

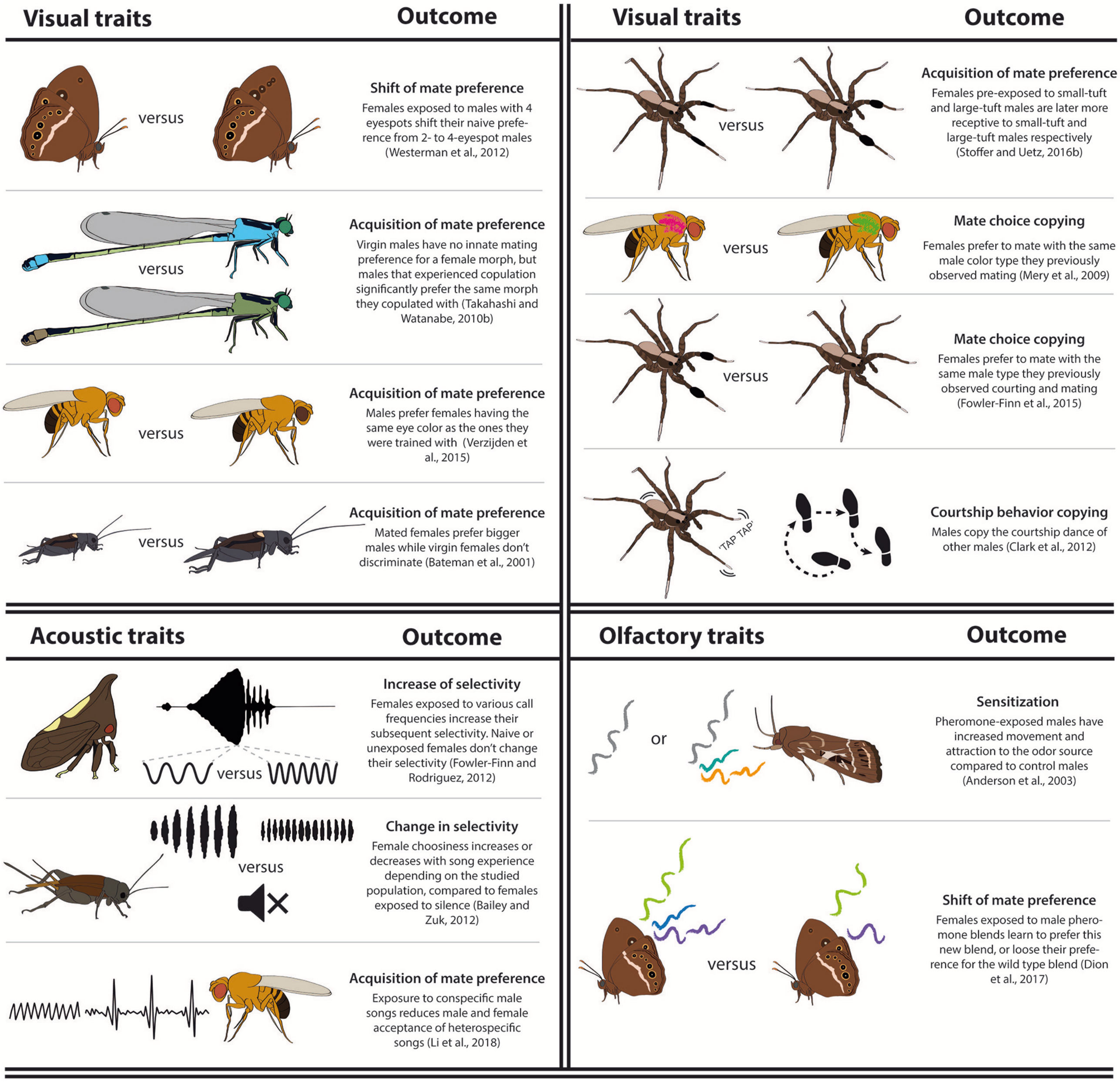
MOLECULAR MECHANISMS OF LEARNING

As detailed above, insects can change their sexual preferences and signaling upon social experiences and exposure to a variety of visual, odor, gustatory, or auditory signals, indicating that sexual behaviors are not fixed but plastic. The underlying molecular mechanisms that control this plasticity, however, are still largely unclear. Below we review a few mechanisms mediating such neural system plasticity.

A social learning experience, such as courtship conditioning, where males experience female rejection in response to courtship, can lead to long-term changes in the behavior of males. This process of long-term memory consolidation in male *Drosophila* appears to depend on a peak of the ecdysteroid hormone, 20E, that appears immediately after the conditioning (Ishimoto et al., 2009).

Insects can learn to prefer (or avoid) a novel visual signal in a mate via early exposure to that signal but mechanisms of plasticity for preference development have only been explored in a non-sexual context. For instance, mRNA of three opsin genes in worker casts of the ant *Camponotus rufipes* increased upon exposure of these ants to daylight, as did volume of the three subneuropils of the optic lobe (including lamina, medulla, and lobula) (Yilmaz et al., 2016). A specific increase in UV and green opsin mRNA was also observed in the moth *Helicoverpa armigera* in response to 6 h exposure to UV light (Yan et al., 2014). These examples suggest that exposure of insects to particular visual signals displayed by the opposite sex could lead to changes in specific opsin expression levels as well as structural changes in the optic lobe, increasing sensitivity to those signals, and perhaps leading to later changes in sexual behaviors and preferences. This remains however to be investigated. Changes in protein expression levels and in cell size, cell number and cell connectivity of higher brain compartments in response to details of color patterns or courtship steps, rather than mere exposure to light of different colors, are also likely taking place but mediating mechanisms are still not known.

Mechanisms of pheromone odor sensitization have been explored to some extent in *Spodoptera* moths. In these experiments males are being briefly pre-exposed to a scent plume containing one or more components of the pheromone blend that increased their sensitization to the odor relative to naïve males (Anderson et al., 2003). The mechanisms that mediate this sensitization involve increases of the specific olfactory receptor expression and odor binding proteins in the antennae a few hours after the exposure (Wan et al., 2015), increased firing responses of the antennae (López et al., 2017)



Shift of mate preference
Females exposed to males with 4 eyespots shift their naive preference from 2- to 4-eyespot males (Westerman et al., 2012)

Acquisition of mate preference
Virgin males have no innate mating preference for a female morph, but males that experienced copulation significantly prefer the same morph they copulated with (Takahashi and Watanabe, 2010b)

Acquisition of mate preference
Males prefer females having the same eye color as the ones they were trained with (Verzijden et al., 2015)

Acquisition of mate preference
Mated females prefer bigger males while virgin females don't discriminate (Bateman et al., 2001)

Acquisition of mate preference
Females pre-exposed to small-tuft and large-tuft males are later more receptive to small-tuft and large-tuft males respectively (Stoffer and Uetz, 2016b)

Mate choice copying
Females prefer to mate with the same male color type they previously observed mating (Mery et al., 2009)

Mate choice copying
Females prefer to mate with the same male type they previously observed courting and mating (Fowler-Finn et al., 2015)

Courtship behavior copying
Males copy the courtship dance of other males (Clark et al., 2012)

Increase of selectivity
Females exposed to various call frequencies increase their subsequent selectivity. Naive or unexposed females don't change their selectivity (Fowler-Finn and Rodriguez, 2012)

Change in selectivity
Female choosiness increases or decreases with song experience depending on the studied population, compared to females exposed to silence (Bailey and Zuk, 2012)

Acquisition of mate preference
Exposure to conspecific male songs reduces male and female acceptance of heterospecific songs (Li et al., 2018)

Sensitization
Pheromone-exposed males have increased movement and attraction to the odor source compared to control males (Anderson et al., 2003)

Shift of mate preference
Females exposed to male pheromone blends learn to prefer this new blend, or loose their preference for the wild type blend (Dion et al., 2017)

FIGURE 2 | Illustrations of learning sexual traits or preferences in insects and spiders. Learning can produce an acquisition or a shift of preference, or a change in the insect selectivity for the sexual signal. These processes can be due to mate choice or sexual signaling copying or to sensitization, which is an increase of the individual attraction to the stimuli upon exposure. All examples illustrate changes in the mate preference function, except for the courtship behavior copying, which illustrates a change in sexual signaling.

and of the odor receptor neurons (Guerrieri et al., 2012), as well as changes in the size of the neural compartment processing the pheromone components in the olfactory lobes (Guerrieri et al., 2012). These physiological and structural changes have been hypothesized to lead to long-term memory of the early odor experience and stable changes in behavior (Anderson et al., 2007; Guerrieri et al., 2012).

Males learn to recognize mated or heterospecific *Drosophila* females thanks to the presence of cuticular hydrocarbons and antiaphrodisiacs transferred by the previous male onto the female's cuticle and reproductive tract (Ejima et al., 2005; Billeter et al., 2009). Recent work has identified neuronal differences across *Drosophila* species that are responsible for species-specific mate preferences regarding a female cuticular pheromone sensed by the legs of males (Seeholzer et al., 2018). It is possible that this conserved neuronal circuit, which is activated differently across species, is plastic and will be later implicated in learning of novel cuticular pheromones within a species, but this remains to be tested.

Drosophila males produce courtship songs by vibrating their wings. The song frequencies are perceived by the tip of the antennae, which detects air particle oscillations, and are processed by the Johnston's organ, at the base of the antennae (Ishikawa and Kamikouchi, 2016). The mechanisms that mediate the female's development of a song preference are still largely unknown but they involve signaling via the main inhibitory neurotransmitter, gamma-Aminobutyric acid (GABA), acting on the GABAA receptor Rdl in specific neurons (Li et al., 2018).

Currently there is limited evidence that learned mate preferences can be transmitted to the next generation in an insect and even more limited understanding regarding mechanisms. Daughters of *B. anynana* females exposed to novel sex pheromone blends show naïve preferences similar to those of their exposed mothers and different from non-exposed naïve individuals (Dion et al., 2017), but the mechanisms mediating the inheritance of this learned preference are unknown. Prolonged (5 day) olfactory conditioning in *Drosophila* was also inherited across two generations, but this work did not test the role of these learned odors on sexual behaviors (Williams, 2016).

MATHEMATICAL MODELS SHOW THAT LEARNING HAS AN EVOLUTIONARY IMPACT

In this section, we first briefly introduce models that assess whether learning itself can evolve and be selected as an evolutionary stable strategy (ESS), i.e., a strategy that if adopted by a population in a given environment cannot be invaded by any alternative strategy that is initially rare. If learning sexual traits is an ESS, then this implies that learning sexual traits is adaptive. Whether learning sexual traits affects their evolution and impacts speciation has also mostly been addressed with theoretical models that we also reviewed in this section.

The first set of models reveal that learning can be selected as an ESS, which is a prerequisite for learning to affect the evolution of sexual signals, mate preferences, and reproductive isolation (reviewed in Galef and Laland, 2005; Vakirtzis, 2011; Verzijden et al., 2012; Dukas, 2013; Servedio and Dukas, 2013; Witte et al., 2015; Head et al., 2016; Kopp et al., 2018; Varela et al., 2018). Overall, for learning to evolve under selection there needs to be genetic variation for learning ability within a species (Mery and Kawecki, 2005). This appears to be the case. An example involves the "rover" and "sitter" alleles at the *foraging* locus of *D. melanogaster* that confer different learning abilities to fly larvae when foraging for food (Mery et al., 2007; Papaj and Snell-Rood, 2007; Mery, 2013).

The second set of models assess whether learning affects the evolution of sexual traits and impacts speciation. These models were originally designed for sexual interactions in vertebrates, but here we focused on those models that can be applied to insects (**Supplementary Table 1**). Most of these models were built on the premise that sexual signals and mate preferences have a genetic basis (e.g., Ritchie, 2000; Shaw, 2000; Noor et al., 2001) that can be modified and be overridden by learning (**Supplementary Table 1**). The models usually focus on one of the following three underlying mechanisms of learning: "learning by sexual imprinting," "learning by copying," and "learning from previous experience" (**Table 3** and **Supplementary Table 1**).

The first group of models focused on imprinting, which occurs when juveniles up to a certain age can learn a sexual preference by observing the phenotypes of surrounding adults (Immelmann, 1975; Head et al., 2016). In insects, the terms "early experience," or "early exposure" to other individuals of the same generation are used instead of "imprinting" (**Table 3**). This is primarily because there is still no data on whether or not insects have a fixed period in development or early adulthood when they can learn a preference from a social experience, as in the case of birds, where the term imprinting was first used (Lorenz, 1935). In insects, sexual imprinting of mate preferences can occur between genetically unrelated individuals of the previous or of the same generation, and is termed oblique or horizontal imprinting, respectively (**Table 3**). Oblique or horizontal imprinting have limited effect on the evolution of sexual preferences and of reproductive isolation, except when spatial structure is taken into account. Spatial structure in models assumes that social learning is only possible between individuals that can perceive each other (Yeh and Servedio, 2015), i.e., that are close in space. Differentiation in social interactions between populations due to spatial isolation is expected to accelerate divergence of sexual preferences, signals, and of reproductive isolation between populations, through coupling of the divergent sexual signals and mate preferences across space (Bailey and Moore, 2012). If populations are exchanging migrants, oblique imprinting of mate preferences cannot produce sympatric speciation (Verzijden et al., 2007), and the populations cannot maintain genetic differentiation in their sexual traits (Yeh and Servedio, 2015). Interestingly, aversive learning of mate preference through oblique imprinting, when individuals learn to avoid a phenotype, was shown to accelerate reproductive isolation and to produce adaptive radiations (Gilman and Kozak, 2015). Finally, imprinting may contribute to reproductive isolation in insects through self-imprinting (i.e., self-referent phenotype matching) that facilitates reinforcement between incipient divergent lineages (Servedio et al., 2009).

Imprinting can also affect the expression of sexual signals, and these learned signals can also contribute to reproductive isolation (Williams and Slater, 1990; Ellers and Slabbekoorn, 2003; Lachlan and Servedio, 2004; Olofsson and Servedio, 2008; Olofsson et al., 2011; Planqué et al., 2014). These models often incorporate a spatial structure, but it is unclear if they can be applicable to insects because they assume that males learn to produce their sexual signals by imitating adults surrounding them during their development, which has so far has been documented only in the wolf spider *Schizocosa ocreata* (Clark et al., 2012, 2015). These models also assume that assortative mating takes place between females and males that have learned to prefer, or express, a similar sexual signal by experiencing it locally, while assortative mating based on similarly preferred and expressed sexual traits does not occur in insects, as far as we know.

A second mechanism of learning sexual behaviors in insects is to copy another individual's mating decision. Insects that mate in groups, such as promiscuous or lekking species [e.g., some species of ants, bees, paper wasps, and butterflies (Litte, 1979; Wickman and Jansson, 1997; Velthuis et al., 2005; Izzo and Tibbetts, 2012; Prato and Soares, 2013)] can modify their mating preference or the production of their own sexual signals by observing the success of other individuals mating. Most work has focused on "mate choice copying" ("MCC" hereafter), which is usually modeled as "positive" such that individuals (usually females) learn to prefer the phenotype of males that they have observed mating earlier (Servedio and Kirkpatrick, 1996; Santos et al., 2017). The copying behavior itself can spread in a population both through direct (Dugatkin and Höglund, 1995; Stöhr, 1998) or indirect (Servedio and Kirkpatrick, 1996; Santos et al., 2017) selection. MCC has direct selective benefits, if it reduces the sampling costs and/or the error rate of mate choice (Dugatkin and Höglund, 1995; Stöhr, 1998; Agrawal, 2001), but also has indirect selective benefits (Kirkpatrick and Dugatkin, 1994; Servedio and Kirkpatrick, 1996; Santos et al., 2017). These indirect benefits arise because females that copy others are more likely to mate with males that are attractive to other females, spreading in the process genes for attractive sons and genes for daughters with the ability to copy others. MCC can also both increase the variance in male sexual signals (Wade and Pruett-Jones, 1990), and erode genetic variance by eliminating novel or rare male signals, even if these males are fitter than the common males in the population (Kirkpatrick and Dugatkin, 1994). However, when biases in learning are present in mate choice copying, such that females are more strongly affected by experiences involving unusual stimuli (e.g., rare male phenotypes) than those involving standard stimuli (e.g., common male phenotypes), MCC can cause novel male signals to sweep through the population even if there is no inherent preference for the novel trait (Agrawal, 2001). Invasion of a novel sexual signal can also occur when "negative" MCC (aversive learning) is modeled, where females learn to avoid males avoided by other females (Santos et al., 2014). While Kirkpatrick and Dugatkin (1994) suggest that MCC may promote or accelerate population divergence, the role of MCC in reproductive isolation and speciation remains an open question (Varela et al., 2018).

A third learning mechanism occurs through previous (so-called "private" or "personal") experiences of either courtship or of actual mating events, during which mate preferences and the expression of sexual signals can be learned (Servedio and Dukas, 2013; Morier-Genoud and Kawecki, 2015). Females learning to prefer local or familiar (previously encountered) males increases the rate of divergence between spatially structured populations (Bailey and Moore, 2012), and also in case of a secondary contact (Servedio and Dukas, 2013). In contrast, when males learn to prefer local or familiar females, population divergence can be reduced because competition for accessing these females increases. Heterospecific males, which seldom meet heterospecific females locally, don't learn to prefer them as much as conspecific males, and keep courting and mating with both types of females (Servedio and Dukas, 2013). When males learn to improve the expression of their sexual signals through repeated courtship events, this accelerates the evolution of the sexual signal, even when the signal is costly, and it favors the emergence and spread of a novel male sexual signal, even in the presence of gene flow (Morier-Genoud and Kawecki, 2015).

Finally, some models compared the fitness advantage, or the likelihood of various mechanisms of mate preference learning to spread as evolutionary stable strategies (ESS). Learned mate preferences from previous encounters with potential mates increase fitness compared to other mate selection mechanisms including threshold-based mate preference (Dubois et al., 2012). Furthermore, the advantage provided by learning increases when variance in the quality among males increases locally, and across space or time (Collins et al., 2006). Depending on associated costs, learning mate preferences either through MCC or through previous personal experience can both coexist as an ESS in mixed populations of females displaying either one or the other learning mechanisms (Dubois et al., 2012).

CONCLUSIONS AND PERSPECTIVES

Experimental work on the role of learning in sexual interactions in insects and spiders is a burgeoning field revealing that the traditional view that insects are small robots with mostly innate, genetically fixed sexual behaviors, is now obsolete. Learning in sexual interactions is the rule rather than the exception in every organism tested so far. Innate, genetically fixed sexual preferences and signals are present only in some species, and are more commonly observed in specific modes of communication such as olfactory signals, whereas learning a sexual preference or the expression of a sexual signal is widespread. Learning also affects the expression of innate sexual traits. Learning is usually assumed to be positive, but it can also be negative (i.e., aversive), as well as biased and context-dependent.

Our review revealed the diversity of terminology used by authors to describe experiments involving learning [**Table 1**, **2**, column "Type of learning (as per the authors)"], which can be confusing and prevent the identification of the underlying mechanisms. Hence, we would like to encourage researchers to provide explicit details of their methods as described in **Tables 1–3** (e.g., the developmental stage at which the learning happens, the sex of the demonstrator and of the insects that learns). This information will specify the processes of learning used by insects, e.g., sensitization, simple exposure, or conditioning, which will help identify the underlying neurophysiological mechanisms involved.

Finally, most scientists assume that learning sexual traits has evolved under selection, as appears to be the case in vertebrates (Morand-Ferron, 2017) and regarding other behaviors in insects (Nieberding et al., 2018), but there is little to no evidence that learning sexual traits affects insect fitness, particularly in the wild. This is perhaps because one of the first definition of learning included adaptation [learning is an "adaptive change in individual behavior as the result of experience;" Thorpe (1963)]. We encourage field work to complement laboratory experiments with ecologically-relevant setups to quantify the adaptive value of learned sexual interactions across insects. Showing the adaptive value of such learning would explain its prevailing presence in such miniature brained, short lived, organisms.

AUTHOR CONTRIBUTIONS

ED, AM, and CN conceived the scope of the review, collected information from the literature, and contributed to the writing and editing of the manuscript. All authors contributed to revisions.

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SUPPLEMENTARY MATERIAL

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REFERENCES

Agrawal, A. F. (2001). The evolutionary consequences of mate copying on male traits. *Behav. Ecol. Sociobiol.* 51, 33–40. doi: 10.1007/s002650100401

Anderson, P., Hansson, B. S., Nilsson, U., Han, Q., Sjoholm, M., Skals, N., et al. (2007). Increased behavioral and neuronal sensitivity to sex pheromone after brief odor experience in a moth. *Chem. Senses* 32, 483–491. doi: 10.1093/chemse/bjm017

Anderson, P., Sadek, M. M., and Hansson, B. S. (2003). Pre-exposure modulates attraction to sex pheromone in a moth. *Chem. Senses* 28, 285–291. doi: 10.1093/chemse/28.4.285

Anderson, P., Sadek, M. M., Larsson, M., Hansson, B. S., and Thöming, G. (2013). Larval host plant experience modulates both mate finding and oviposition choice in a moth. *Anim. Behav.* 85, 1169–1175. doi: 10.1016/j.anbehav.2013.03.002

Anton, S., Evengaard, K., Barrozo, R. B., Anderson, P., and Skals, N. (2011). Brief predator sound exposure elicits behavioral and neuronal long-term sensitization in the olfactory system of an insect. *PNAS* 108, 3401–3405. doi: 10.1073/pnas.1008840108

Archer, M. S., and Elgar, M. A. (1999). Female preference for multiple partners: sperm competition in the hide beetle, *Dermestes maculatus* (DeGeer). *Anim. Behav.* 58, 669–675. doi: 10.1006/anbe.1999.1172

Bailey, N. W., Gray, B., and Zuk, M. (2010). Acoustic experience shapes alternative mating tactics and reproductive investment in male field crickets. *Curr. Biol.* 20, 845–849. doi: 10.1016/j.cub.2010.02.063

Bailey, N. W., Hoskins, J. L., Green, J., and Ritchie, M. G. (2013). Measuring same-sex sexual behaviour: the influence of the male social environment. *Anim. Behav.* 86, 91–100. doi: 10.1016/j.anbehav.2013.04.016

Bailey, N. W., and Macleod, E. (2014). Socially flexible female choice and premating isolation in field crickets (*Teleogryllus* spp.). *J. Evol. Biol.* 27, 170–180. doi: 10.1111/jeb.12285

Bailey, N. W., and Moore, A. J. (2012). Runaway sexual selection without genetic correlations: social environments and flexible mate choice initiate and enhance the Fisher process. *Evolution* 66, 2674–2684. doi: 10.1111/j.1558-5646.2012.01647.x

Bailey, N. W., and Zuk, M. (2008). Acoustic experience shapes female mate choice in field crickets. *Proc. Biol. Sci.* 275, 2645–2650. doi: 10.1098/rspb.2008.0859

Bailey, N. W., and Zuk, M. (2009). Field crickets change mating preferences using remembered social information. *Biol. Lett.* 5, 449–451. doi: 10.1098/rsbl.2009.0112

Bailey, N. W., and Zuk, M. (2012). Socially flexible female choice differs among populations of the Pacific field cricket: geographical variation in the interaction coefficient psi (Psi). *Proc. Biol. Sci.* 279, 3589–3596. doi: 10.1098/rspb.2012.0631

Balaban-Feld, J., and Valone, T. J. (2017). Prior information and social experience influence male reproductive decisions. *Behav. Ecol.* 28, 1376–1383. doi: 10.1093/beheco/arx105

Barron, A. B., Hebets, E. A., Cleland, T. A., Fitzpatrick, C. L., Hauber, M. E., and Stevens, J. R. (2015). Embracing multiple definitions of learning. *Trends Neurosci.* 38, 405–407. doi: 10.1016/j.tins.2015.04.008

Barrows, E. M. (1975). Individually distinctive odors in an invertebrate. *Behav. Biol.* 15, 57–64. doi: 10.1016/S0091-6773(75)92079-9

Bateman, P. W. (1998). Mate preference for novel partners in the cricket *Gryllus bimaculatus*. *Ecol. Entomol.* 23, 473–475. doi: 10.1046/j.1365-2311.1998.00156.x

Bateman, P. W., and Fleming, P. A. (2006). Males are selective too: mating, but not courtship, with sequential females influences choosiness in male field crickets (*Gryllus bimaculatus*). *Behav. Ecol. Sociobiol.* 59, 577–581. doi: 10.1007/s00265-005-0083-y

Bateman, P. W., Gilson, L. N., and Ferguson, J. W. H. (2001). Male size and sequential mate preference in the cricket *Gryllus bimaculatus*. *Anim. Behav.* 61, 631–637. doi: 10.1006/anbe.2000.1617

Billeter, J.-C., Atallah, J., Krupp, J. J., Millar, J. G., and Levine, J. D. (2009). Specialized cells tag sexual and species identity in *Drosophila melanogaster*. *Nature* 461:987. doi: 10.1038/nature08495

Bretman, A., Fricke, C., Hetherington, P., Stone, R., and Chapman, T. (2010). Exposure to rivals and plastic responses to sperm competition in *Drosophila melanogaster*. *Behav. Ecol.* 21, 317–321. doi: 10.1093/beheco/arp189

Carballo, M., Baldenegro, F., Bollatti, F., Peretti, A. V., and Aisenberg, A. (2017). No pain, no gain: male plasticity in burrow digging according to female rejection in a sand-dwelling wolf spider. *Behav. Processes* 140, 174–180. doi: 10.1016/j.beproc.2017.05.007

Chittka, L., and Niven, J. (2009). Are bigger brains better? *Curr. Biol.* 19, R995–R1008. doi: 10.1016/j.cub.2009.08.023

Clark, D. L., Kizer Zeeff, C., Sabovodny, G., Hollenberg, A., Roberts, J. A., and Uetz, G. W. (2015). The role of social experience in eavesdropping by male wolf spiders (Lycosidae). *Anim. Behav.* 106, 89–97. doi: 10.1016/j.anbehav.2015.05.001

Clark, D. L., Roberts, J. A., and Uetz, G. W. (2012). Eavesdropping and signal matching in visual courtship displays of spiders. *Biol. Lett.* 8, 375–378. doi: 10.1098/rsbl.2011.1096

Collins, E. J., McNamara, J. M., and Ramsey, D. M. (2006). Learning rules for optimal selection in a varying environment: mate choice revisited. *Behav. Ecol.* 17, 799–809. doi: 10.1093/beheco/arl008

Dagaeff, A.-C., Pocheville, A., Nöbel, S., Loyau, A., Isabel, G., and Danchin, E. (2016). *Drosophila* mate copying correlates with atmospheric pressure in a speed learning situation. *Anim. Behav.* 121, 163–174. doi: 10.1016/j.anbehav.2016.08.022

Danci, A., Hrabar, M., Ikoma, S., Schaefer, P. W., and Gries, G. (2013). Learning provides mating opportunities for males of a parasitoid wasp. *Entomol. Exp. Appl.* 149, 229–240. doi: 10.1111/eea.12129

Danci, A., Inducil, C., Takács, S., Schaefer, P. W., and Gries, G. (2014). Mechanism of mate detection in parasitoid wasps: sound and vibratory cues change with the developmental progress of future mates inside host pupal cases. *Physiol. Entomol.* 39, 292–303. doi: 10.1111/phen.12075

de Jager, M. L., and Ellis, A. G. (2014). Costs of deception and learned resistance in deceptive interactions. *Proc. Biol. Sci.* 281:20132861. doi: 10.1098/rspb.2013.2861

Dion, E., Pui, L. X., and Monteiro, A. (2017). Early-exposure to new sex pheromone blend alters mate preference in female butterflies and in their offspring. *Biorxiv [Preprint]*. doi: 10.1101/214635

Dubois, F., Drullion, D., and Witte, K. (2012). Social information use may lead to maladaptive decisions: a game theoretic model. *Behav. Ecol.* 23, 225–231. doi: 10.1093/beheco/arr179

Dugatkin, L. A., and Höglund, J. (1995). Delayed breeding and the evolution of mate copying in lekking species. *J. Theor. Biol.* 174, 261–267. doi: 10.1006/jtbi.1995.0097

Dukas, R. (2004). Male fruit flies learn to avoid interspecific courtship. *Behav. Ecol.* 15, 695–698. doi: 10.1093/beheco/arh068

Dukas, R. (2005). Experience improves courtship in male fruit flies. *Anim. Behav.* 69, 1203–1209. doi: 10.1016/j.anbehav.2004.08.012

Dukas, R. (2006). Learning in the context of sexual behaviour in insects. *Anim. Biol.* 56, 125–141. doi: 10.1163/157075606777304258

Dukas, R. (2008a). Evolutionary biology of insect learning. *Annu. Rev. Entomol.* 53, 145–160. doi: 10.1146/annurev.ento.53.103106.093343

Dukas, R. (2008b). Learning decreases heterospecific courtship and mating in fruit flies. *Biol. Lett.* 4, 645–647. doi: 10.1098/rsbl.2008.0437

Dukas, R. (2009). Dynamics of learning in the context of courtship in *Drosophila persimilis* and *D. pseudoobscura. Anim. Behav.* 77, 253–259. doi: 10.1016/j.anbehav.2008.10.010

Dukas, R. (2010). Causes and consequences of male-male courtship in fruit flies. *Anim. Behav.* 80, 913–919. doi: 10.1016/j.anbehav.2010.08.017

Dukas, R. (2013). Effects of learning on evolution: robustness, innovation and speciation. *Anim. Behav.* 85, 1023–1030. doi: 10.1016/j.anbehav.2012.12.030

Dukas, R., and Baxter, C. M. (2014). Mate choosiness in young male fruit flies. *Behav. Ecol.* 25, 549–552. doi: 10.1093/beheco/aru020

Dukas, R., and Dukas, L. (2012). Learning about prospective mates in male fruit flies: effects of acceptance and rejection. *Anim. Behav.* 84, 1427–1434. doi: 10.1016/j.anbehav.2012.09.010

Ejima, A., Smith, B. P., Lucas, C., Levine, J. D., and Griffith, L. C. (2005). Sequential learning of pheromonal cues modulates memory consolidation in trainer-specific associative courtship conditioning. *Curr. Biol.* 15, 194–206. doi: 10.1016/j.cub.2005.01.035

Ejima, A., Smith, B. P., Lucas, C., Van der Goes van Naters, W., Miller, C. J., Carlson, J. R., et al. (2007). Generalization of courtship learning in *Drosophila* is mediated by cis-vaccenyl acetate. *Curr. Biol.* 17, 599–605. doi: 10.1016/j.cub.2007.01.053

Ellers, J., and Slabbekoorn, H. (2003). Song divergence and male dispersal among bird populations: a spatially explicit model testing the role of vocal learning. *Anim. Behav.* 65, 671–681. doi: 10.1006/anbe.2003.2081

Filice, D. C. S., and Long, T. A. F. (2017). Phenotypic plasticity in female mate choice behavior is mediated by an interaction of direct and indirect genetic effects in *Drosophila melanogaster*. *Ecol. Evol.* 7, 3542–3551. doi: 10.1002/ece3.2954

Fincke, O. M., Fargevieille, A., and Schultz, T. D. (2007). Lack of innate preference for morph and species identity in mate-searching *Enallagma* damselflies. *Behav. Ecol. Sociobiol.* 61, 1121–1131. doi: 10.1007/s00265-006-0345-3

Fowler-Finn, K. D., Cruz, D. C., and Rodríguez, R. L. (2017). Local population density and group composition influence the signal-preference relationship in *Enchenopa* treehoppers (Hemiptera: Membracidae). *J. Evol. Biol.* 30, 13–25. doi: 10.1111/jeb.12994

Fowler-Finn, K. D., and Rodríguez, R. L. (2012a). The evolution of experience-mediated plasticity in mate preferences. *J. Evol. Biol.* 25, 1855–1863. doi: 10.1111/j.1420-9101.2012.02573.x

Fowler-Finn, K. D., and Rodríguez, R. L. (2012b). Experience-mediated plasticity in mate preference: mating assurance in a variable environment. *Evolution* 66, 459–468. doi: 10.1111/j.1558-5646.2011.01446.x

Fowler-Finn, K. D., Sullivan-Beckers, L., Runck, A. M., and Hebets, E. A. (2015). The complexities of female mate choice and male polymorphisms: elucidating the role of genetics, age, and mate-choice copying. *Curr. Zool.* 61, 1015–1035. doi: 10.1093/czoolo/61.6.1015

Gailey, D. A., Jackson, F. R., and Siegel, R. W. (1982). Male courtship in Drosophila: the conditioned response to immature males and its genetic control. *Genetics* 102, 771–782.

Galef, B. G., and Laland, K. N. (2005). Social Learning in Animals: empirical studies and theoretical models. *Bioscience* 55, 489–499. doi: 10.1641/0006-3568(2005)055[0489:SLIAES]2.0.CO;2

Germain, M., Blanchet, S., Loyau, A., and Danchin, É. (2016). Mate-choice copying in *Drosophila melanogaster:* impact of demonstration conditions and male–male competition. *Behav. Processes* 125, 76–84. doi: 10.1016/j.beproc.2016.02.002

Gilman, R. T., and Kozak, G. M. (2015). Learning to speciate: the biased learning of mate preferences promotes adaptive radiation. *Evolution* 69, 3004–3012. doi: 10.1111/evo.12797

Griffith, L. C., and Ejima, A. (2009). Courtship learning in *Drosophila melanogaster*: diverse plasticity of a reproductive behavior. *Learn. Mem.* 16, 743–750. doi: 10.1101/lm.956309

Guerrieri, F., Gemeno, C., Monsempes, C., Anton, S., Jacquin-Joly, E., Lucas, P., et al. (2012). Experience-dependent modulation of antennal sensitivity and input to antennal lobes in male moths (*Spodoptera littoralis*) pre-exposed to sex pheromone. *J. Exp. Biol.* 215, 2334–2341. doi: 10.1242/jeb.060988

Harari, A. R., Zahavi, T., and Thiery, D. (2011). Fitness cost of pheromone production in signaling female moths. *Evolution* 65, 1572–1582. doi: 10.1111/j.1558-5646.2011.01252.x

Head, M. L., Jennions, M. D., and Zajitschek, S. R. K. (2016). Sexual selection: incorporating non-genetic inheritance. *Curr. Opin. Behav. Sci.* 12, 129–137. doi: 10.1016/j.cobeha.2016.10.005

Hebets, E. (2003). Subadult experience influences adult mate choice in an arthropod: exposed female wolf spiders prefer males of a familiar phenotype. *PNAS* 100, 13390–13395. doi: 10.1073/pnas.2333262100

Hebets, E. (2007). Subadult female experience does not influence species recognition in the wolf spider *schizocosa uetzi* stratton 1997. *J. Arachnol.* 35, 1–10. doi: 10.1636/S05-76.1

Hebets, E., and Sullivan-Beckers, L. (2010). "Mate choice and learning," in *Encyclopedia of Animal Behavior,* ed. M.D.B.a.J. Moore. (Amsterdam: Elsevier), 389–393. doi: 10.1016/B978-0-08-045337-8.00364-8

Hebets, E. A., and Vink, C. J. (2007). Experience leads to preference: experienced females prefer brush-legged males in a population of syntopic wolf spiders. *Behav. Ecol.* 18, 1010–1020. doi: 10.1093/beheco/arm070

Hedwig, B. (2016). *Insect Hearing and Acoustic Communication*. Cambridge, UK: Springer.

Hoefler, C. D., Moore, J. A., Reynolds, K. T., and Rypstra, A. L. (2010). The effect of experience on male courtship and mating behaviors in a cellar spider. *Am. Midl. Nat.* 163, 255–268. doi: 10.1674/0003-0031-163.2.255

Immelmann, K. (1975). Ecological significance of imprinting and early learning. *Annu. Rev. Ecol. Syst.* 6, 15–37. doi: 10.1146/annurev.es.06.110175.000311

Ishikawa, Y., and Kamikouchi, A. (2016). Auditory system of fruit flies. *Hearing Res.* 338, 1–8. doi: 10.1016/j.heares.2015.10.017

Ishimoto, H., Sakai, T., and Kitamoto, T. (2009). Ecdysone signaling regulates the formation of long-term courtship memory in adult *Drosophila melanogaster*. *PNAS* 106, 6381–6386. doi: 10.1073/pnas.0810213106

Izzo, A. S., and Tibbetts, E. A. (2012). Spotting the top male: sexually selected signals in male *Polistes dominulus* wasps. *Anim. Behav.* 83, 839–845. doi: 10.1016/j.anbehav.2012.01.005

Johnson, J. C. (2005). Cohabitation of juvenile females with mature males promotes sexual cannibalism in fishing spiders. *Behav. Ecol.* 16, 269–273. doi: 10.1093/beheco/arh152

Judge, K. A., Tran, K.-C., and Gwynne, D. T. (2010). The relative effects of mating status and age on the mating behaviour of female field crickets. *Can. J. Zool.* 88, 219–223. doi: 10.1139/Z09-139

Kasumovic, M. M., Hall, M. D., and Brooks, R. C. (2012). The juvenile social environment introduces variation in the choice and expression of sexually selected traits. *Ecol. Evol.* 2, 1036–1047. doi: 10.1002/ece3.230

Kirkpatrick, M., and Dugatkin, L. A. (1994). Sexual selection and the evolutionary effects of copying mate choice. *Behav. Ecol. Sociobiol.* 34, 443–449. doi: 10.1007/BF00167336

Kopp, M., Servedio, M. R., Mendelson, T. C., Safran, R. J., Rodriguez, R. L., Hauber, M. E., et al. (2018). Mechanisms of assortative mating in speciation with gene flow: connecting theory and empirical research. *Am. Nat.* 191, 1–20. doi: 10.1086/694889

Kujtan, L., and Dukas, R. (2009). Learning magnifies individual variation in heterospecific mating propensity. *Anim. Behav.* 78, 549–554. doi: 10.1016/j.anbehav.2009.05.026

Lachlan, R. F., and Servedio, M. R. (2004). Song learning accelerates allopatric speciation. *Evolution* 58, 2049–2063. doi: 10.1111/j.0014-3820.2004.tb00489.x

Li, X., Ishimoto, H., and Kamikouchi, A. (2018). Auditory experience controls the maturation of song discrimination and sexual response in Drosophila. *Elife* 7:e34348. doi: 10.7554/eLife.34348

Li, Y.-Y., Yu, J.-F., Lu, Q., Xu, J., and Ye, H. (2014). Female and male moths display different reproductive behavior when facing new versus previous mates. *PLoS ONE* 9:e109564. doi: 10.1371/journal.pone.0109564

Litte, M. (1979). *Mischocyttarus flavitarsis* in Arizona: social and nesting biology of a polistine wasp. *Zeitschrift für Tierpsychologie* 50, 282–312. doi: 10.1111/j.1439-0310.1979.tb01033.x

López, S., Guerrero, A., Bleda, M. J., and Quero, C. (2017). Short-term peripheral sensitization by brief exposure to pheromone components in *Spodoptera littoralis*. *J. Comp. Physiol. A* 203, 973–982. doi: 10.1007/s00359-017-1205-5

Lorenz, K. (1935). Der Kumpan in der Umwelt des Vogels. *J. für Ornithologie* 83, 289–413. doi: 10.1007/BF01905572

Loyau, A., Blanchet, S., Van Laere, P., Clobert, J., and Danchin, E. (2012). When not to copy: female fruit flies use sophisticated public information to avoid mated males. *Sci. Rep.* 2:768. doi: 10.1038/srep00768

McRobert, S. P., and Tompkins, L. (1988). Two consequences of homosexual courtship performed by *Drosophila melanogaster* and *Drosophila affinis* males. *Evolution* 42, 1093–1097. doi: 10.1111/j.1558-5646.1988.tb02528.x

Mery, F. (2013). Natural variation in learning and memory. *Curr. Opin. Neurobiol.* 23, 52–56. doi: 10.1016/j.conb.2012.09.001

Mery, F., Belay, A. T., So, A. K., Sokolowski, M. B., and Kawecki, T. J. (2007). Natural polymorphism affecting learning and memory in *Drosophila*. *PNAS* 104, 13051–13055. doi: 10.1073/pnas.0702923104

Mery, F., and Kawecki, T. J. (2005). A cost of long-term memory in Drosophila. *Science* 308:1148. doi: 10.1126/science.1111331

Mery, F., Varela, S. A. , Danchin, É., Blanchet, S., Parejo, D., Coolen, I., et al. (2009). Public versus personal information for mate copying in an invertebrate. *Curr. Biol.* 19, 730–734. doi: 10.1016/j.cub.2009.02.064

Miller, M. N., and Fincke, O. M. (1999). Cues for mate recognition and the effect of prior experience on mate recognition in *Enallagma* damselflies. *J. Insect Behav.* 12, 801–814. doi: 10.1023/A:1020957110842

Miller, M. N., and Fincke, O. M. (2004). Mistakes in sexual recognition among sympatric *Zygoptera* vary with time of day and color morphism (Odonata: Coenagrionidae). *Int. J. Odonatol* 7, 471–491. doi: 10.1080/13887890.2004.9748233

Morand-Ferron, J. (2017). Why learn? The adaptive value of associative learning in wild populations. *Curr. Opin. Behav. Sci* 16, 73–79. doi: 10.1016/j.cobeha.2017.03.008

Morand-Ferron, J., and Quinn, J. L. (2015). The evolution of cognition in natural populations. *Trends Cogn. Sci.* 19, 235–237. doi: 10.1016/j.tics.2015.03.005

Morier-Genoud, R., and Kawecki, T. J. (2015). The effect of learning on the evolution of new courtship behavior: a simulation model. *Curr. Zool.* 61, 1062–1072. doi: 10.1093/czoolo/61.6.1062

Nieberding, C. M., Van Dyck, H., and Chittka, L. (2018). Adaptive learning in non-social insects: from theory to field work, and back. *Curr. Opin. Insect Sci.* 27, 75–81. doi: 10.1016/j.cois.2018.03.008

Nöbel, S., Allain, M., Isabel, G., and Danchin, E. (2018a). Mate copying in *Drosophila melanogaster* males. *Anim. Behav.* 141, 9–15. doi: 10.1016/j.anbehav.2018.04.019

Nöbel, S., Danchin, E., and Isabel, G. (2018b). Mate-copying for a costly variant in *Drosophila melanogaster* females. *Behav. Ecol.* 29, 1150–1156. doi: 10.1093/beheco/ary095

Noor, M. A., Grams, K. L., Bertucci, L. A., and Reiland, J. (2001). Chromosomal inversions and the reproductive isolation of species. *PNAS* 98, 12084–12088. doi: 10.1073/pnas.221274498

Odeen, A., and Moray, C. M. (2008). *Drosophila melanogaster* virgins are more likely to mate with strangers than familiar flies. *Naturwissenschaften* 95, 253–256. doi: 10.1007/s00114-007-0314-3

Olofsson, H., Frame, A. M., and Servedio, M. R. (2011). Can reinforcement occur with a learned trait? *Evolution* 65, 1992–2003. doi: 10.1111/j.1558-5646.2011.01286.x

Olofsson, H., and Servedio, M. R. (2008). Sympatry affects the evolution of genetic versus cultural determination of song. *Behav. Ecol.* 19, 596–604. doi: 10.1093/beheco/arn002

Pan, Y., and Baker, B. S. (2014). Genetic identification and separation of innate and experience-dependent courtship behaviors in *Drosophila*. *Cell* 156, 236–248. doi: 10.1016/j.cell.2013.11.041

Papaj, D. R., and Snell-Rood, E. C. (2007). Memory flies sooner from flies that learn faster. *PNAS* 104, 13539–13540. doi: 10.1073/pnas.0706165104

Planqué, R., Britton, N. F., and Slabbekoorn, H. (2014). On the maintenance of bird song dialects. *J. Math. Biol.* 68, 505–531. doi: 10.1007/s00285-012-0632-8

Prato, M., and Soares, A. E. E. (2013). Production of sexuals and mating frequency in the stingless bee *Tetragonisca angustula* (Latreille) (Hymenoptera, Apidae). *Neotrop. Entomol.* 42, 474–482. doi: 10.1007/s13744-013-0154-0

Proffit, M., Khallaf, M. A., Carrasco, D., Larsson, M. C., and Anderson, P. (2015). 'Do you remember the first time?' Host plant preference in a moth is modulated by experiences during larval feeding and adult mating. *Ecol. Lett.* 18, 365–374. doi: 10.1111/ele.12419

Rebar, D., Barbosa, F., and Greenfield, M. D. (2016). Acoustic experience influences male and female pre- and postcopulatory behaviors in a bushcricket. *Behav. Ecol.* 27, 434–443. doi: 10.1093/beheco/arv171

Rebar, D., and Rodríguez, R. L. (2013). Genetic variation in social influence on mate preferences. *Proc. Biol. Sci.* 280: 20130803. doi: 10.1098/rspb.2013.0803

Rebar, D., and Rodriguez, R. L. (2016). Males adjust their signalling behaviour according to experience of male signals and male-female signal duets. *J. Evol. Biol.* 29:766. doi: 10.1111/jeb.12825

Rebar, D., Zuk, M., and Bailey, N. W. (2011). Mating experience in field crickets modifies pre- and postcopulatory female choice in parallel. *Behav. Ecol.* 22, 303–309. doi: 10.1093/beheco/arq195

Ritchie, M. G. (2000). The inheritance of female preference functions in a mate recognition system. *Proc. Biol. Sci.* 267, 327–332. doi: 10.1098/rspb.2000.1004

Robledo, N., Arzuffi, R., and Reyes-Prado, H. (2018). Modification of behavioral response in *Copitarsia decolora* (Lepidoptera: Noctuidae) due to pre-exposure to sex pheromone and host plant volatiles. *Fla. Entomol.* 101, 69–73. doi: 10.1653/024.101.0113

Rutledge, J. M., Miller, A., and Uetz, G. W. (2010). Exposure to multiple sensory cues as a juvenile affects adult female mate preferences in wolf spiders. *Anim. Behav.* 80, 419–426. doi: 10.1016/j.anbehav.2010.05.027

Rutledge, J. M., and Uetz, G. W. (2014). Juvenile experience and adult female mating preferences in two closely related *Schizocosa* species. *J. Arachnol.* 42, 170–177. doi: 10.1636/J14-10.1

Sadek, M. M., von Wowern, G., Löfstedt, C., Rosén, W.-Q., and Anderson, P. (2012). Modulation of the temporal pattern of calling behavior of female *Spodoptera littoralis* by exposure to sex pheromone. *J. Insect Physiol.* 58, 61–66. doi: 10.1016/j.jinsphys.2011.09.016

Saleem, S., Ruggles, P. H., Abbott, W. K., and Carney, G. E. (2014). Sexual experience enhances *Drosophila melanogaster* male mating behavior and success. *PLoS ONE* 9:e96639. doi: 10.1371/journal.pone.0096639

Santos, M., Matos, M., and Varela, S. A. M. (2014). Negative public information in mate choice copying helps the spread of a novel trait. *Am. Nat.* 184, 658–672. doi: 10.1086/678082

Santos, M., Sapage, M., Matos, M., and Varela, S. A. M. (2017). Mate-choice copying: a fitness-enhancing behavior that evolves by indirect selection. *Evolution* 71, 1456–1464. doi: 10.1111/evo.13235

Schlechter-Helas, J., Schmitt, T., and Peschke, K. (2012). Learning individual signatures: rove beetle males discriminate unreceptive females by cuticular hydrocarbon patterns. *Anim. Behav.* 84, 369–376. doi: 10.1016/j.anbehav.2012.05.003

Seeholzer, L. F., Seppo, M., Stern, D. L., and Ruta, V. (2018). Evolution of a central neural circuit underlies *Drosophila* mate preferences. *Nature* 559, 564–569. doi: 10.1038/s41586-018-0322-9

Servedio, M. R. (2015). Advances on the interplay of learning and sexual selection. *Curr. Zool.* 61, 1004–1007. doi: 10.1093/czoolo/61.6.1004

Servedio, M. R., and Dukas, R. (2013). Effects on population divergence of within-generational learning about prospective mates. *Evolution* 67, 2363–2375. doi: 10.1111/evo.12127

Servedio, M. R., and Kirkpatrick, M. (1996). The evolution of mate choice copying by indirect selection. *Am. Nat.* 148, 848–867. doi: 10.1086/285959

Servedio, M. R., Sæther, S. A., and Sætre, G.-P. (2009). Reinforcement and learning. *Evol. Ecol.* 23, 109–123. doi: 10.1007/s10682-007-9188-2

Shaw, K. L. (2000). Interspecific genetics of mate recognition: inheritance of female acoustic preference in Hawaiian crickets. *Evolution* 54, 1303–1312. doi: 10.1111/j.0014-3820.2000.tb00563.x

Shaw, K. L., and Herlihy, D. P. (2000). Acoustic preference functions and song variability in the Hawaiian cricket *Laupala cerasina*. *Proc. Biol. Sci.* 267, 577–584. doi: 10.1098/rspb.2000.1040

Silvegren, G., Lofstedt, C., and Qi Rosen, W. (2005). Circadian mating activity and effect of pheromone pre-exposure on pheromone response rhythms in the moth *Spodoptera littoralis*. *J. Insect Physiol.* 51, 277–286. doi: 10.1016/j.jinsphys.2004.11.013

Smith, B. H. (1983). Recognition of female kin by male bees through olfactory signals. *PNAS* 80, 4551–4553. doi: 10.1073/pnas.80.14.4551

Stejskal, K., Streinzer, M., Dyer, A., Paulus, H. F., and Spaethe, J. (2015). Functional significance of labellum pattern variation in a sexually deceptive orchid (*Ophrys heldreichii*): evidence of individual signature learning effects. *PLoS ONE* 10:e0142971. doi: 10.1371/journal.pone.0142971

Stelinski, L., Il'ichev, A., and Gut, L. (2006). Antennal and behavioral responses of virgin and mated oriental fruit moth (Lepidoptera: Tortricidae) females to their sex pheromone. *Ann. Entomol. Soc. Am.* 99, 898–904. doi: 10.1603/0013-8746(2006)99[898:AABROV]2.0.CO;2

Stockton, D. G., Martini, X., and Stelinski, L. (2017). Male psyllids differentially learn in the context of copulation. *Insects* 8:16. doi: 10.3390/insects8010016

Stoffer, B., and Uetz, G. W. (2015). The effects of social experience with varying male availability on female mate preferences in a wolf spider. *Behav. Ecol. Sociobiol.* 69, 927–937. doi: 10.1007/s00265-015-1904-2

Stoffer, B., and Uetz, G. W. (2016a). Social experience affects female mate preferences for a visual trait in a wolf spider. *Behav. Ecol.* 27, 252–261. doi: 10.1093/beheco/arv143

Stoffer, B., and Uetz, G. W. (2016b). Tuft size matters: the effects of adult visual social experience on female mate preferences in a wolf spider. *Behav. Ecol. Sociobiol.* 70, 2211–2221. doi: 10.1007/s00265-016-2225-9

Stoffer, B., and Uetz, G. W. (2017). The effects of experience with different courtship modalities on unimodal and multimodal preferences in a wolf spider. *Anim. Behav.* 123, 187–196. doi: 10.1016/j.anbehav.2016.10.033

Stöhr, S. (1998). Evolution of mate-choice copying: a dynamic model. *Anim. Behav.* 55, 893–903. doi: 10.1006/anbe.1997.0674

Suckling, D. M., Stringer, L. D., Jimenez-Perez, A., Walter, G. H., Sullivan, N., and El-Sayed, A. M. (2018). With or without pheromone habituation: possible differences between insect orders? *Pest Manag. Sci.* 74, 1259–1264. doi: 10.1002/ps.4828

Sullivan-Beckers, L., and Hebets, E. A. (2011). Modality-specific experience with female feedback increases the efficacy of courtship signalling in male wolf spiders. *Anim. Behav.* 82, 1051–1057. doi: 10.1016/j.anbehav.2011.07.040

Sullivan-Beckers, L., and Hebets, E. A. (2014). Tactical adjustment of signalling leads to increased mating success and survival. *Anim. Behav.* 93, 111–117. doi: 10.1016/j.anbehav.2014.04.021

Svensson, E. I., Eroukhmanoff, F., Karlsson, K., Runemark, A., and Brodin, A. (2010). A role for learning in population divergence of mate preferences. *Evolution* 64, 3101–3113. doi: 10.1111/j.1558-5646.2010.01085.x

Svensson, E. I., Runemark, A., Verzijden, M. N., and Wellenreuther, M. (2014). Sex differences in developmental plasticity and canalization shape population divergence in mate preferences. *Proc. Biol. Sci.* 281:20141636. doi: 10.1098/rspb.2014.1636

Swanger, E., and Zuk, M. (2015). Cricket responses to sexual signals are influenced more by adult than juvenile experiences. *J. Insect Behav.* 28, 328–337. doi: 10.1007/s10905-015-9504-6

Takahashi, Y., and Watanabe, M. (2010a). Diurnal changes in male mate preference to female dimorphism in *Ischnura senegalensis* (Rambur) (Zygoptera: Coenagrionidae). *Odonatologica* 39, 159–162.

Takahashi, Y., and Watanabe, M. (2010b). Mating experience affecting male discrimination between sexes and female morphs in *Ischnura senegalensis* (Rambur) (Zygoptera: Coenagrionidae). *Odonatologica* 39, 47–56.

Takahashi, Y., and Watanabe, M. (2011). Male mate choice based on ontogenetic colour changes of females in the damselfly *Ischnura senegalensis*. *J. Ethol.* 29, 293–299. doi: 10.1007/s10164-010-0257-6

Tan, C. K., Lovlie, H., Greenway, E., Goodwin, S. F., Pizzari, T., and Wigby, S. (2013). Sex-specific responses to sexual familiarity, and the role of olfaction in *Drosophila*. *Proc. Biol. Sci.* 280:20131691. doi: 10.1098/rspb.2013.1691

Thöming, G., Larsson Mattias, C., Hansson Bill, S., and Anderson, P. (2013). Comparison of plant preference hierarchies of male and female moths and the impact of larval rearing hosts. *Ecology* 94, 1744–1752. doi: 10.1890/12-0907.1

Thorpe, W. H. (1963). *Learning and Instinct in Animals.* Methuen, MA: Methuen Publishing Ltd.

Tinghitella, R. M. (2014). Male and female crickets modulate their courtship behaviour depending on female experience with mate availability. *Anim. Behav.* 91, 9–15. doi: 10.1016/j.anbehav.2014.02.022

Vakirtzis, A. (2011). Mate choice copying and nonindependent mate choice: a critical review. *Ann. Zool. Fenn.* 48, 91–107. doi: 10.5735/086.048.0202

van Gossum, H., De Bruyn, L., and Stoks, R. (2005). Reversible switches between male–male and male–female mating behaviour by male damselflies. *Biol. Lett.* 1, 268–270. doi: 10.1098/rsbl.2005.0315

van Gossum, H., Stoks, R., and Bruyn, L. D. (2001). Reversible frequency–dependent switches in male mate choice. *Proc. Biol. Sci.* 268, 83–85. doi: 10.1098/rspb.2000.1333

Varela, S. A. M., Matos, M., and Schlupp, I. (2018). The role of mate-choice copying in speciation and hybridization. *Biol. Rev. Camb. Philos. Soc.* 93, 1304–1322. doi: 10.1111/brv.12397

Velthuis, H. H. W., Koedam, D., and Imperatriz-Fonseca, V. L. (2005). The males of *Melipona* and other stingless bees, and their mothers. *Apidologie* 36, 169–185. doi: 10.1051/apido:2005014

Verzijden M. N., Lachlan Robert, F., and Servedio Maria, R. (2007). Female mate-choice behavior and sympatric speciation. *Evolution* 59, 2097–2108. doi: 10.1111/j.0014-3820.2005.tb00920.x

Verzijden, M. N., Abbott, J. K., von Philipsborn, A. C., and Loeschcke, V. (2015). Male *Drosophila melanogaster* learn to prefer an arbitrary trait associated with female mating status. *Curr. Zool.* 61, 1036–1042. doi: 10.1093/czoolo/61.6.1036

Verzijden, M. N., and Svensson, E. I. (2016). Interspecific interactions and learning variability jointly drive geographic differences in mate preferences. *Evolution* 70, 1896–1903. doi: 10.1111/evo.12982

Verzijden, M. N., ten Cate, C., Servedio, M. R., Kozak, G. M., Boughman, J. W., and Svensson, E. I. (2012). The impact of learning on sexual selection and speciation. *Trends Ecol. Evol.* 27, 511–519. doi: 10.1016/j.tree.2012.05.007

Wade, M. J., and Pruett-Jones, S. G. (1990). Female copying increases the variance in male mating success. *PNAS* 87, 5749–5753. doi: 10.1073/pnas.87.15.5749

Wagner, W. E., Smeds, M. R., and Wiegmann, D. D. (2001). Experience affects female responses to male song in the variable field cricket *Gryllus lineaticeps* (Orthoptera, Gryllidae). *Ethology* 107, 769–776. doi: 10.1046/j.1439-0310.2001.00700.x

Wan, X., Qian, K., and Du, Y. (2015). Synthetic pheromones and plant volatiles alter the expression of chemosensory genes in *Spodoptera exigua*. *Sci. Rep.* 5:17320. doi: 10.1038/srep17320

Wcislo, W. T. (1987). The role of learning in the mating biology of a sweat bee *Lasioglossum zephyrum* (Hymenoptera: Halictidae). *Behav. Ecol. Sociobiol.* 20, 179–185. doi: 10.1007/BF00299731

Weinstein, A. M., Davis, B. J., Menz, M. H. M., Dixon, K. W., and Phillips, R. D. (2016). Behaviour of sexually deceived ichneumonid wasps and its implications for pollination in *Cryptostylis* (Orchidaceae). *Biol. J. Linn. Soc.* 119, 283–298. doi: 10.1111/bij.12841

Westerman, E. L., Chirathivat, N., Schyling, E., and Monteiro, A. (2014). Mate preference for a phenotypically plastic trait is learned, and may facilitate preference-phenotype matching. *Evolution* 68, 1661–1670. doi: 10.1111/evo.12381

Westerman, E. L., Hodgins-Davis, A., Dinwiddie, A., and Monteiro, A. (2012). Biased learning affects mate choice in a butterfly. *PNAS* 109, 10948–10953. doi: 10.1073/pnas.1118378109

Westerman, E. L., and Monteiro, A. (2013). Odour influences whether females learn to prefer or to avoid wing patterns of male butterflies. *Anim. Behav.* 86, 1139–1145. doi: 10.1016/j.anbehav.2013.09.002

Wickman, P.-O., and Jansson, P. (1997). An estimate of female mate searching costs in the lekking butterfly *Coenonympha pamphilus*. *Behav. Ecol. Sociobiol.* 40, 321–328. doi: 10.1007/s002650050348

Wilder, S. M., and Rypstra, A. L. (2008). Prior encounters with the opposite sex affect male and female mating behavior in a wolf spider (Araneae, Lycosidae). *Behav. Ecol. Sociobiol.* 62, 1813–1820. doi: 10.1007/s00265-008-0610-8

Williams, J. M., and Slater, P. J. B. (1990). Modelling bird song dialects: the influence of repertoire size and numbers of neighbours. *J. Theor. Biol.* 145, 487–496. doi: 10.1016/S0022-5193(05)80483-7

Williams, Z. M. (2016). Transgenerational influence of sensorimotor training on offspring behavior and its neural basis in *Drosophila*. *Neurobiol. Learn. Mem.* 131, 166–175. doi: 10.1016/j.nlm.2016.03.023

Witte, K., Kniel, N., and Kureck, I. M. (2015). Mate-choice copying: Status quo and where to go. *Curr. Zool.* 61, 1073–1081. doi: 10.1093/czoolo/61.6.1073

Xu, J., and Wang, Q. (2009). A polyandrous female moth discriminates against previous mates to gain genetic diversity. *Anim. Behav.* 78, 1309–1315. doi: 10.1016/j.anbehav.2009.09.028

Yan, S., Zhu, J., Zhu, W., Zhang, X., Li, Z., Liu, X., et al. (2014). The expression of three opsin genes from the compound eye of *Helicoverpa armigera* (Lepidoptera: Noctuidae) is regulated by a circadian clock, light conditions and nutritional status. *PLoS ONE* 9:e111683. doi: 10.1371/journal.pone.0111683

Yeh, D. J., and Servedio, M. R. (2015). Reproductive isolation with a learned trait in a structured population. *Evolution* 69, 1938–1947. doi: 10.1111/evo.12688

Yilmaz, A., Lindenberg, A., Albert, S., Grubel, K., Spaethe, J., Rossler, W., et al. (2016). Age-related and light-induced plasticity in opsin gene expression and in primary and secondary visual centers of the nectar-feeding ant *Camponotus rufipes*. *Dev. Neurobiol.* 76, 1041–1057. doi: 10.1002/dneu.22374

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Toward Testing for Multimodal Perception of Mating Signals

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Many mating signals consist of multimodal components that need decoding by several sensory modalities on the receiver's side. For methodological and conceptual reasons, the communicative functions of these signals are often investigated only one at a time. Likewise, variation of single signal traits are frequently correlated by researchers with senders' quality or receivers' behavioral responses. Consequently, the two classic and still dominating hypotheses regarding the communicative meaning of multimodal mating signals postulate that different components either serve as back-up messages or provide multiple meanings. Here we discuss how this conceptual dichotomy might have hampered a more integrative, perception encompassing understanding of multimodal communication: neither the multiple message nor the back-up signal hypotheses address the possibility that multimodal signals are integrated neurally into one percept. Therefore, when studying multimodal mating signals, we should be aware that they can give rise to multimodal percepts. This means that receivers can gain access to additional information inherent in combined signal components only ("the whole is something different than the sum of its parts"). We review the evidence for the importance of multimodal percepts and outline potential avenues for discovery of multimodal percepts in animal communication.

Keywords: multimodal percepts, sensory integration, mating signals, emergent properties, perceptual or sensory binding, mate choice, animal communication

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MULTIMODALITY IS A CHARACTERISTIC OF MANY MATING SIGNALS

To attract mates, many animals simultaneously signal in more than one modality. Signals with components in more than one modality are multimodal signals (for glossary see **Box 1**). Multimodal signals are taxonomically widespread: flies court with a display that combines visual, acoustic, vibratory, and chemical signal components. Frog calls are often accompanied by visually conspicuous vocal sac movements and/or water surface vibrations (**Figure 1A**). In both these examples, signal variants in single vs. combined modalities result in different behavioral reactions of receivers (Narins et al., 2005; Bretman et al., 2011). A closer look at other taxonomic groups shows more examples: Many species of birds show complex, rhythmic visual displays during singing (Williams, 2001; Dalziell et al., 2013; Ullrich et al., 2016), fish grunt and quiver (Estramil et al., 2014; de Jong et al., 2018), spiders and grasshoppers have visual-vibratory courtship displays (Stafstrom and Hebets, 2013; Kozak and Uetz, 2016; Vedenina and Shestakov, 2018), and some

Box 1 | Glossary.

Term	Definition
Multi-component signals	Displays with >1 component, but all received within one sensory modality
Multiple signals	Multiple signals within the same modality but eliciting separate responses (in same or different receivers)
Multimodal signal	>1 component and >1 sensory modality
Cross-sensory integration	Information from different sensory modalities is integrated at higher sensory levels
Multimodal perception	>1 component and >1 sensory modality are integrated to form 1 multimodal percept
Perceptual binding (also: sensory binding)	Process through which multimodal cues or signals (in communication) are grouped by the brain to belonging to one object or being one signal
Unity assumption	Well-studied sensory phenomenon in humans: two stimuli close in space or time are assumed to belong to the same object, at basis of ventriloquism effect

bats sing songs while fanning odors from a wing-pouch toward their intended mates (Voigt et al., 2008). Although multimodal mating signals are common, the single modalities are mostly studied apart (often owing to the technical specializations required to conduct the research). Consequently, description, analyses, and experimental tests of the form and function of animal mating signals have mostly been unimodal. This changed in the 1990's when behavioral ecologists started to draw attention to multi-component and multimodal signaling (Møller and Pomiankowski, 1993; Partan and Marler, 1999; Candolin, 2003) and how (multimodal) receiver psychology might excert in itself selective pressures on signal evolution (Rowe, 1999). Experimental studies in diverse fields, e.g., aposematic signaling (Rowe and Guilford, 1996) started to investigate how multimodal signals might be integrated by receivers. The research field on mating signals conceptually took a different direction by focusing more on signal content (discussed in Hebets et al., 2016). Perhaps owing to the field's strong focus on function rather than behavioral mechanisms, influential reviews at the time (Møller and Pomiankowski, 1993; Johnstone, 1996; Candolin, 2003) centered around two signal-content centered hypotheses:

(1) Multimodal signals are backup signals—the same message (e.g., species identity) is given in multiple sensory modalities and if one channel is blocked, a potential receiver can still receive the intended message.
(2) Multimodal signals convey multiple messages—simultaneously emitted signal components in multiple modalities contain different information content (e.g., one component conveys species identity and the other the intention to mate).

Both these hypotheses overly focus on message coding on the sender's rather than on the perceptual processes on the receiver's side (Hebets et al., 2016). Growing empirical and theoretical insights show that this focus on signal production should be complemented by studying receivers' perceptual mechanisms to fully characterize the complexity of (multimodal) animal communication (Rowe, 1999; Hebets and Papaj, 2005; Partan and Marler, 2005; Starnberger et al., 2014; Halfwerk and Slabbekoorn, 2015; Hebets et al., 2016; Ryan et al., 2019). Importantly, documenting multimodal signals is but a first step; it will not reveal whether receivers process the signal components of the different modalities separately (as suggested by the backup-signal and multiple-message hypotheses) or integrate them into one single multimodal percept with perhaps a qualitatively different meaning. It is this process of multimodal perception in the narrow sense, i.e., higher sensory integration of multimodal input (see **Box 1**) for which we aim to raise further awareness in the context of mate signaling, because sensory integration resulting in multimodal percepts can lead to receiver responses that may fundamentally differ from responses to unimodal components. Multimodal perception is intensively researched in human psychobiology and the cognitive neurosciences [see e.g., (Spence, 2011; Stein, 2012; Chen and Vroomen, 2013)] where cross-sensory integration has been demonstrated in different vertebrates species, for example, non-human primates (Maier et al., 2008; Perrodin et al., 2015), cats (Meredith and Stein, 1996), and birds (Whitchurch and Takahashi, 2006).

A multimodal percept *sensu strictu* arises, whenever the central nervous system integrates simultaneous information of separate sensory modalities so that the resulting percept is qualitatively different from the sum of the properties of its components. A multimodal percept is thus contingent on the combined input of the involved modalities and absent when only a single component is present. A multimodal percept involves a concurrent larger, smaller or unique neural and/or behavioral response to multimodal vs. unimodal stimulation (Stein et al., 2014). This means that a multimodal percept can convey unique messages, a notion that is different from the "back-up" or "multiple-messages" concepts. This has important consequences for empirical work because some components when studied in isolation may be inadvertently dismissed as irrelevant in mate attraction or mate choice (**Figure 1**). *In extremis* unimodal experimental presentations can lead to false negatives, wrongly dismissing the ecological function and evolutionary importance of a particular sexual display. With this perspective paper our foremost aim is to raise awareness for multimodal perception in the context of mating signals by first reviewing how it is identified in human psychology and the neurosciences and then discuss selected candidate examples of similar phenomena in animals.

MULTIMODAL PERCEPTION: EVIDENCE FROM PSYCHOPHYSICAL STUDIES IN HUMANS

Multisensory processing in humans is evident in daily life and perhaps best illustrated by multisensory illusions (Shams et al., 2000). For example, in the *double-flash illusion*, subjects will perceive two visual light flashes during the presentation

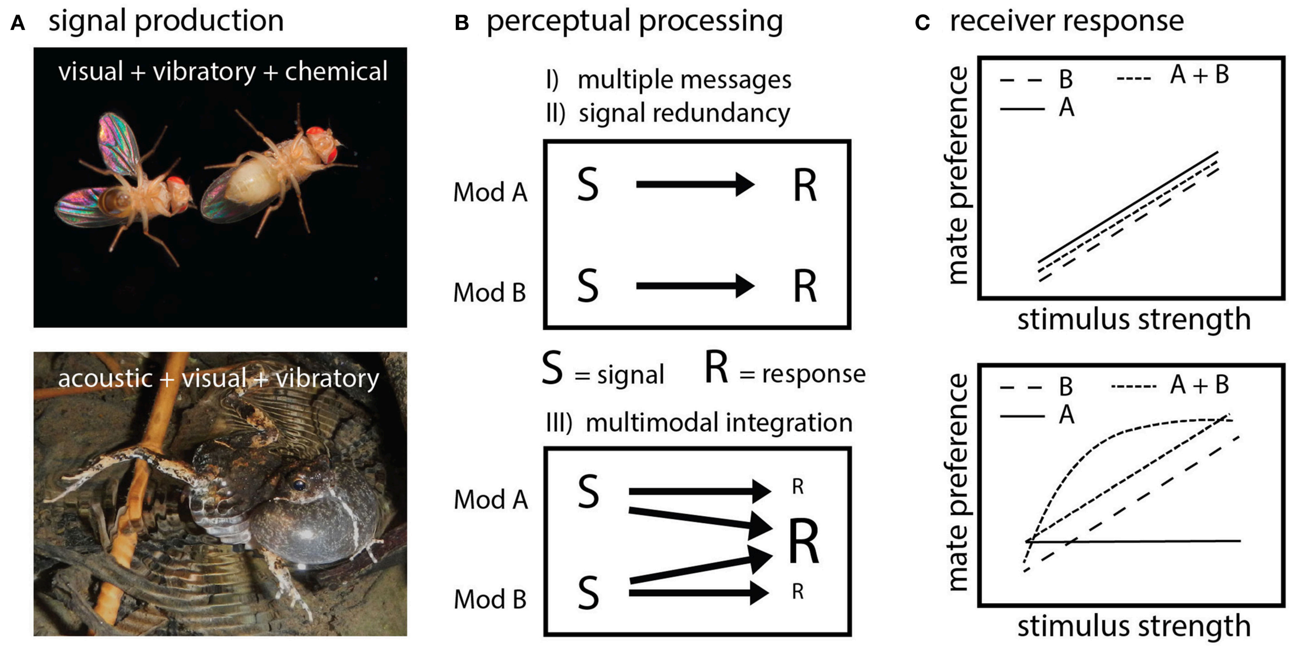

FIGURE 1 | Multimodal signal production, perception and response. **(A)** Many mating displays involve components that are produced in and received through different physical modalities. Drosophila fruit flies displays combine chemical signal components, with visual and vibratory wing components (top panel). Tungara frogs produce sounds, while simultaneously inflating a visually conspicuous vocal sac and making water surface vibrations (bottom panel). **(B)** Multimodal components are either independently processed by the receiver's brain (top panel), as proposed by the multiple messages and backup signal hypotheses, or are integrated at higher levels of sensory processing, or so-called centers of multisensory integration (bottom panel). Mod A refers to one signal modality (e.g., visual) and Mod B refers to another signal modality (e.g., acoustic). S refers to signal component as produced by the sender, R refers to receiver response. **(C)** Expected receiver responses to either backup, multiple messages or models incorporating multisensory integration illustrated by using preference function plots (x-axis depicting a relative/normalized stimulus dimension/trait value e.g., calling rate or plumage hue in relation to preference strength on the y-axis). The two classic hypotheses of backup and multiple messages predict that the response to signal components presented in isolation does not differ from the response to their combined presentation (top panel). The difference between the two classic models being that response to A in isolation is either similar (backup) or different (multiple messages) from response to B. During multisensory integration responses to unimodal vs. multimodal presentation differ: they could increase, decrease or show non-linear characteristics. For illustration, two cases are plotted where the response to multimodal presentation either reflects a linear or non-linear (curved line) process.

of a single flash if the latter is accompanied by two quick repetitions of a sound. The illusion demonstrates that auditory information can alter the perception of visual information and that the information from the two modalities is combined to one percept. The *double-flash illusion* exemplifies the process of cross-sensory integration (also referred to as "perceptual or sensory binding," see **Box 1**). Ventriloquist effects on the other hand demonstrate how visual modifies auditory information (Stein, 2012). If concurrent audio and visual cues come from different locations (= conflicting spatial information) the visual dominates the auditory cue. Puppeteers use this effect when speaking with unmoving lips while simultaneously moving the puppet's mouth, leaving the audience with the impression that this is the source of the sound. Another striking example of multisensory integration in speech perception is the McGurk-effect (McGurk and Macdonald, 1976). Mismatching lip movements during speech production can alter what subjects hear: for example when human subjects see a video of a person's face articulating the syllables "ga-ga-ga" while hearing a person saying "ba-ba-ba," most native speakers of American English will report hearing "da-da-da." The simultaneous presentation of mismatched visual and auditory speech cues that provide ambiguous information can lead to a novel percept that is different from the physical properties of the two original stimuli—the visual information changes what subjects are hearing (McGurk and Macdonald, 1976). These phenomena result from the process of perceptual binding, which is the capacity to group different stimuli as belonging to the same source (see **Box 1**). Cues that arrive from the same direction, or at similar time intervals likely belong together and are thus grouped as such (Stein, 2012).

MULTIMODAL PERCEPTION IN NON-HUMAN ANIMALS

There are plenty of examples of multisensory processing in animals—predators, such as bats, locate their prey faster if they can use information in more than one modality (Rhebergen et al., 2015; Leavell et al., 2018). Bumblebees learn new food sources faster if they can combine visual and weakly electric signals of flowers (Clarke et al., 2013). Birds learn to avoid predators quicker if vision is combined with sound (Rojas et al., 2018).

Such changes in behavioral output during multi- vs. unimodal presentations are potential, but not conclusive evidence for a multimodal percept *sensu strictu*. These changes can also arise from other cognitive processes, like faster reaction times arising from alerting effects (Rowe, 1999). However, well-designed experiments can demonstrate sensory integration resulting in multimodal percepts. Many prey animals use toxic substances as primary defense and signal these with conspicuous signals (e.g., the bright yellow and red warning colorations of many invertebrates or poison frogs). Naïve young chickens will peck equally often at novel yellow or green colored food grains but prefer green and avoid yellow in the presence of the chemical cue pyrazine (a substance that makes many insects unpalatable). Here multisensory integration results in a new emergent percept that is different from the sum of its parts: pyrazine odors trigger a color aversion not shown in the absence of these odors (Rowe and Guilford, 1996). Multimodal integration is also evidenced by ventriloquism effects in frogs and birds (Narins and Smith, 1986; Narins et al., 2005; Feenders et al., 2017). In an operant task simulating a "temporal-order-judgment-task" used to test sensory binding in humans (involving the presentations of simple tones and light flashes), starlings received a food reward if they identified which of two different lights was activated first by pecking an associated response key. The starlings showed better discrimination when the visual stimuli were preceded or followed by a sound (Feenders et al., 2017). Because both sounds either before ("leading") or after ("trailing") the visual presentation improved visual temporal resolution, an alerting function of the sound can be excluded. Trailing (or leading) sounds seem to perceptually attract the second light flash, thereby perceptually increasing the gap between the two visual stimuli, thus improving their discrimination. In this example, the starlings were trained in a foraging context, but clearly, multimodal perceptual grouping would improve identifying and locating competitors and mates in situations with high sensory information load like frogs' mate advertising choruses (**Figure 2**). So how strong is the case for multimodal perception in a sexually selected context?

MULTIMODAL PERCEPTION IN MATE ATTRACTION AND RESOURCE DEFENSE

Numerous observations show that identification of potential mates or rivals requires information from more than one sensory modality: female fish approach potential mates faster or exclusively if presented with signals in two modalities e.g., vision and sound (Estramil et al., 2014) or vision and pheromones (McLennan, 2003). Fruitflies only react to conspecifics if stimulated in at least two modalities (interestingly so, in any combination of the acoustic, volatile, visual, gustatory or vibratory components) by species-specific signals (Bretman et al., 2011). These examples are highly suggestive of multimodal percepts, but as discussed above, a stronger response to a multimodal signal is not conclusive demonstration of multimodal perception yet. Fortunately, alternative explanations, such as increased attention resulting from alerting or additive effects on motivation, can often be excluded on the behavioral level with suitable experimental designs.

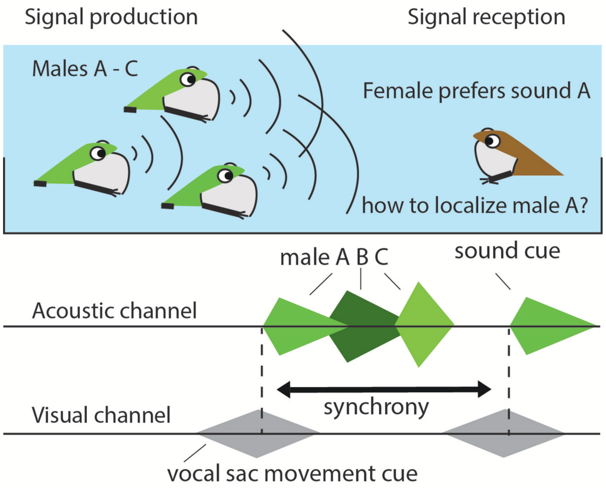

FIGURE 2 | Multimodal perception requires comparing cues across sensory systems. A female frog that prefers to mate with a male that makes (to her) the most attractive sound can face the problem of not being able to locate that male in dense mating chorus. By focusing on the synchrony in sound production and the movements of a male's water surface waves or vocal sac she may be able to pick out her mate. Synchrony can e.g., be assessed by focusing on the timing in intensity between multimodal cues, as illustrated, or on the onset or offset of cues.

A first example is provided by a study of tungara frogs. Males can produce two different call elements, a whine and a chuck. Females will only react to a chuck when it shortly follows the whine. Experimentally increasing the temporal gap between the call elements reduces the attractiveness of the playback to female frogs. However, when a robot frog inflates and deflates its vocal sac during the silent gaps in the unattractive audio playbacks females will prefer the combined audio-visual over the audio-only stimulus. The visual cue thus perceptually binds the two acoustic cues together (Taylor and Ryan, 2013). Inflating the vocal sac prior to or after the gap between the acoustic elements does not restore mate choice, excluding increased attention, discrimination or memorability as alternative explanations (Rowe, 1999). Cross-modal perceptual binding has also been demonstrated to aid females to locate and choose males in other taxonomic groups, for example spiders and birds (e.g., Lombardo et al., 2008; Kozak and Uetz, 2016).

Another example, that shows how multimodal percepts not only help locating a sender, but lead to a qualitative change in judgment compared to a unimodal signal, concerns the multimodal displays shown by duetting avian species. Duets have an important function in joined territory defense and pairbonding (Hall, 2009). Although the next example concerns males' and females' joined breeding territory defense rather than mate attraction, we discuss it in this section for its methodology and because it provides an experimental demonstration of how an avian multimodal display can be crossmodally integrated. Duetting magpie-larks often produce synchronized visual wing waving movements during joined singing (Rek and Magrath, 2017). Combining taxidermic robotic birds with acoustic playbacks revealed that adding visual cues changed the interpretation of auditory cues. During unimodal audio-presentations, behavioral responses were weaker during solo than duet playbacks. During multimodal presentations, adding two wing-waving birds always caused a strong response, whereas adding one wing-waving bird always a weak response, regardless of whether the audio was playing back solo or duet singing. The authors did not set out to test for multimodal percepts in this study but a functional question (whether pseudo-duets are deceptive) and interpret their findings that receivers weigh visual information stronger than auditory information. We would expand this interpretation by suggesting that the observed perceptual weighing indicates cross-sensory binding: The crucial observation here is that adding a single wing-waving bird weakened the previously stronger response shown to duet singing in the audio-only condition—an example of a response that differs from "the sum of its parts." We would argue that, akin to the "*double-flash illusion*" that triggers humans to "see" two flashes when hearing two sounds, the birds that previously heard a duet are now perhaps tricked into "hearing" only one singer when seeing only one bird displaying. In the experiment, the robobirds tricked the receivers, but in real life, cross-modal comparisons would enable receivers to detect the deceptive "pseudo-duets" sometimes used by single singers when out of sight successfully mimicking the structure and complexity of two duetting birds (Rek and Magrath, 2017).

USING ECOLOGICAL VARIATION IN SPACE AND TIME TO TEST FOR MULTIMODAL PERCEPTION AND COMMUNICATION

In this last section we want to place multimodal mating signals in their ecological context since both signal production and perception are affected by social and environmental factors. Thus, for complete characterization of the function of multimodal signals we also need to study the *when* and *how* of signal production on the sender's side, where intended signal receivers are located in relation to the signaler in time and space, habitats' transmission properties (often varying even at a small scale) as well as the processes of reception and perception. An additional dimension of an ecological vs. a laboratory context is the potentially higher number of possible receivers of mating signals: often these are next to mates and rivals also eavesdropping predators (Ratcliffe and Nydam, 2008; Rhebergen et al., 2015).

As discussed above, ventriloquism effects based on sensory binding require temporal and/or spatial proximity (Narins et al., 2005). It is thus crucial, whether signal components of multimodal displays are produced synchronously or asynchronously (temporally and spatially). Many frogs call by in- and deflating a vocal sac which incidentally creates synchronous water-borne vibratory signal components (Halfwerk et al., 2014a). Wolf spiders on the other hand can use one set of legs for drumming and wave another set in the air to create visual signals, not being constrained by mechanical linkage between signal components (Uetz and Roberts, 2002). However, synchrony of the production of signal components can still (and often will) disintegrate during transmission. Light for example transmits a million times faster than airborne sound. The components of a synchronously produced audio-visual mating display will therefore arrive with a temporal lag that increases with distance. Multimodal perception could help detecting senders: synchronously produced visual and acoustic signal components arriving from the same direction likely belong to the same sender and by perceptually binding the acoustic cue to a visual cue, receivers might be able to locate their preferred mate (**Figure 2**), as seems indeed the case in diverse species, e.g., frogs, spiders, and birds (see examples above).

Manipulating either the temporal or the spatial configuration of the signal components can thus be used in field tests. By delaying the timing of water surface vibrations in relation to the timing of the airborne sound, male tungara frogs were tricked into perceiving their rival as displaying from a location outside of their territory (Halfwerk et al., 2014b). Likewise, the synchrony between signal components can be manipulated to assess whether females use temporal cues during multimodal perceptual binding (**Figure 2**). Changing the location from which different signal components are broadcast to receivers may also reveal whether spatial cues are also important for binding (Lombardo et al., 2008; Kozak and Uetz, 2016). Future work can make use of the fast technological progress in audio-video presentations [for review and caveats see Chouinard-Thuly et al. (2017)] or combining acoustic or chemical playbacks accompanied by robots to present different signal components synchronously or asynchronously (Rek and Magrath, 2017; Stange et al., 2017).

CONCLUSIONS

With this brief perspective we hope to have raised interest and awareness regarding potential presence of multimodal signals in mating contexts and the importance of studying perception to understand their function. Supporting previous appeals to integrate cognitive processes on the receiver's side into the study of animal communication ["receiver psychology" (Rowe, 1999; Bateson and Healy, 2005; Ryan et al., 2019)], we hope to have shown that adding the question as to how receivers integrate multiple signal components from different modalities into integrated percepts might add an important dimension to studying multimodal mating signals. Well-designed behavioral experiments have already demonstrated how stimuli of two modalities are coupled via perceptual binding, which can eventually lead to a multimodal percept. However, to date, this process has been predominantly studied in contexts unrelated to mate choice (e.g., foraging or predator-prey interactions) but their increasing documentation across contexts and taxa suggests that the same perceptual processes will also apply to (some) mating signals. A perception-orientated approach can thus shed new light on the discussion of multiple-messages vs. backup-signals. When co-occurring signals in two or more modalities are perceptually integrated the behavioral and evolutionary implications may be different than when those signals are processed in parallel. Multimodal integration provides thus an additional hypothesis with its own predictions regarding message meaning that might need testing when studying multimodal mating signals. Ignoring this possibility can yield misleading results regarding the relative importance of the different signal components when only unimodal tests are conducted—a crucial component could look irrelevant for mate choice when tested in isolation.

AUTHOR CONTRIBUTIONS

WH and KR conceived the idea for the review. JV, RS, EM, CS, WH, and KR contributed to literature search and writing.

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REFERENCES

Bateson, M., and Healy, S. D. (2005). Comparative evaluation and its implications for mate choice. *Trends Ecol. Evol.* 20, 659–664. doi: 10.1016/j.tree.2005.08.013

Bretman, A., Westmancoat, J. D., Gage, M. J. G., and Chapman, T. (2011). Males use multiple, redundant cues to detect mating rivals. *Curr. Biol.* 21, 617–622. doi: 10.1016/j.cub.2011.03.008

Candolin, U. (2003). The use of multiple cues in mate choice. *Biol. Rev.* 78, 575–595. doi: 10.1017/S1464793103006158

Chen, L. H., and Vroomen, J. (2013). Intersensory binding across space and time: a tutorial review. *Atten. Percept. Psychophys.* 75, 790–811. doi: 10.3758/s13414-013-0475-4

Chouinard-Thuly, L., Gierszewski, S., Rosenthal, G. G., Reader, S. M., Rieucau, G., Woo, K. L., et al. (2017). Technical and conceptual considerations for using animated stimuli in studies of animal behavior. *Cur. Zool.* 63, 5–19. doi: 10.1093/cz/zow104

Clarke, D., Whitney, H., Sutton, G., and Robert, D. (2013). Detection and learning of floral electric fields by bumblebees. *Science.* 340, 66–69. doi: 10.1126/science.1230883

Dalziell, A. H., Peters, R. A., Cockburn, A., Dorland, A. D., Maisey, A. C., and Magrath, R. D. (2013). Dance choreography is coordinated with song repertoire in a complex avian display. *Curr. Biol.* 23, 1132–1135. doi: 10.1016/j.cub.2013.05.018

de Jong, K., Amorim, M. C. P., Fonseca, P. J., and Heubel, K. U. (2018). Noise affects multimodal communication during courtship in a marine fish. *Front. Ecol. Evol.* 6:113. doi: 10.3389/fevo.2018.00113.

Estramil, N., Bouton, N., Verzijden, M. N., Hofker, K., Riebel, K., and Slabbekoorn, H. (2014). Cichlids respond to conspecific sounds but females exhibit no phonotaxis without the presence of live males. *Ecol. Freshw. Fish.* 23, 305–312. doi: 10.1111/eff.12081

Feenders, G., Kato, Y., Borzeszkowski, K. M., and Klump, G. M. (2017). Temporal ventriloquism effect in European starlings: evidence for two parallel processing pathways. *Behav. Neurosci.* 131, 337–347. doi: 10.1037/bne0000200

Halfwerk, W., Jones, P. L., Taylor, R. C., Ryan, M. J., and Page, R. A. (2014a). Risky ripples allow bats and frogs to eavesdrop on a multisensory sexual display. *Science.* 343, 413–416. doi: 10.1126/science.1244812

Halfwerk, W., Page, R. A., Taylor, R. C., Wilson, P. S., and Ryan, M. J. (2014b). Crossmodal comparisons of signal components allow for relative distance assessment. *Curr. Biol.* 24, 1751–1755. doi: 10.1016/j.cub.2014.05.068

Halfwerk, W., and Slabbekoorn, H. (2015). Pollution going multimodal: the complex impact of the human-altered sensory environment on animal perception and performance. *Biol. Lett.* 11:20141051. doi: 10.1098/rsbl.2014.1051

Hall, M. L. (2009). A review of vocal duetting in birds. *Adv. Stud. Behav.* 40, 67–121. doi: 10.1016/s0065-3454(09)40003-2

Hebets, E. A., Barron, A. B., Balakrishnan, C. N., Hauber, M. E., Mason, P. H., and Hoke, K. L. (2016). A systems approach to animal communication. *Proc. R. Soci. B Biol. Sci.* 283:20152889. doi: 10.1098/rspb.2015.2889

Hebets, E. A., and Papaj, D. R. (2005). Complex signal function: developing a framework of testable hypotheses. *Behav. Ecol. Sociobiol.* 57, 197–214. doi: 10.1007/s00265-004-0865-7

Johnstone, R. A. (1996). Multiple displays in animal communication: 'Backup signals' and 'multiple messages'. *Phil. Trans. R. Soc. B.* 351, 329–338. doi: 10.1098/rstb.1996.0026

Kozak, E. C., and Uetz, G. W. (2016). Cross-modal integration of multimodal courtship signals in a wolf spider. *Anim. Cogn.* 19, 1173–1181. doi: 10.1007/s10071-016-1025-y

Leavell, B. C., Rubin, J. J., McClure, C. J. W., Miner, K. A., Branham, M. A., and Barber, J. R. (2018). Fireflies thwart bat attack with multisensory warnings. *Sci. Adv.* 4:eaat6601. doi: 10.1126/sciadv.aat6601

Lombardo, S. R., Mackey, E., Tang, L., Smith, B. R., and Blumstein, D. T. (2008). Multimodal communication and spatial binding in pied currawongs (*Strepera graculina*). *Anim. Cogn.* 11, 675–682. doi: 10.1007/s10071-008-0158-z

Maier, J. X., Chandrasekaran, C., and Ghazanfar, A. A. (2008). Integration of bimodal looming signals through neuronal coherence in the temporal lobe. *Curr. Biol.* 18, 963–968. doi: 10.1016/j.cub.2008.05.043

McGurk, H., and Macdonald, J. (1976). Hearing lips and seeing voices. *Nature.* 264, 746–748. doi: 10.1038/264746a0

McLennan, D. A. (2003). The importance of olfactory signals in the gasterosteid mating system: sticklebacks go multimodal. *Biol. J. Linnean Soc.* 80, 555–572. doi: 10.1111/j.1095-8312.2003.00254.x

Meredith, M. A., and Stein, B. E. (1996). Spatial determinants of multisensory integration in cat superior colliculus neurons. *J. Neurophysiol.* 75, 1843–1857. doi: 10.1152/jn.1996.75.5.1843

Møller, A. P., and Pomiankowski, A. (1993). Why have birds got multiple sexual ornaments. *Behav. Ecol. Sociobiol.* 32, 167–176. doi: 10.1007/BF00173774

Narins, P. M., Grabul, D. S., Soma, K. K., Gaucher, P., and Hodl, W. (2005). Cross-modal integration in a dart-poison frog. *Proc. Natl. Acad. Sci. U.S.A.* 102, 2425–2429. doi: 10.1073/pnas.0406407102

Narins, P. M., and Smith, S. L. (1986). Clinal variation in alduran advertisement calls: basis for acoustic isolation? *Behav. Ecol. Sociobiol.* 19, 135–141. doi: 10.1007/BF00299948

Partan, S., and Marler, P. (1999). Communication goes multimodal. *Science.* 283, 1272–1273. doi: 10.1126/science.283.5406.1272

Partan, S. R., and Marler, P. (2005). Issues in the classification of multimodal communication signals. *Am. Nat.* 166, 231–245. doi: 10.1086/431246

Perrodin, C., Kayser, C., Logothetis, N. K., and Petkov, C. I. (2015). Natural asynchronies in audiovisual communication signals regulate neuronal multisensory interactions in voice-sensitive cortex. *Proc. Natl. Acad. Sci. U.S.A.* 112, 273–278. doi: 10.1073/pnas.1412817112

Ratcliffe, J. M., and Nydam, M. L. (2008). Multimodal warning signals for a multiple predator world. *Nature.* 455, 96–U59. doi: 10.1038/nature07087

Rek, P., and Magrath, R. D. (2017). Deceptive vocal duets and multimodal display in a songbird. *Proc. R. Soc. B.* 284:20171774. doi: 10.1098/rspb.2017.1774

Rhebergen, F., Taylor, R. C., Ryan, M. J., Page, R. A., and Halfwerk, W. (2015). Multimodal cues improve prey localization under complex environmental conditions. *Proc. R. Soc. Lond. B Biol. Sci.* 282:20151403. doi: 10.1098/rspb.2015.1403

Rojas, B., Burdfield-Steel, E., De Pasqual, C., Gordon, S., Hernández, L., Mappes, J., et al. (2018). Multimodal aposematic signals and their emerging role in mate attraction. *Front. Ecol. Evol.* 6:93. doi: 10.3389/fevo.2018.00093

Rowe, C. (1999). Receiver psychology and the evolution of multicomponent signals. *Anim. Behav.* 58, 921–931. doi: 10.1006/anbe.1999.1242

Rowe, C., and Guilford, T. (1996). Multiple colour aversions in domestic chicks triggered by pyrazine odours of insect warning colours. *Nature.* 383, 520–522. doi: 10.1038/383520a0

Ryan, M. J., Page, R. A., Hunter, K. L., and Taylor, R. C. (2019). 'Crazy love': nonlinearity and irrationality in mate choice. *Anim. Behav.* 147, 189–198. doi: 10.1016/j.anbehav.2018.04.004

Shams, L., Kamitani, Y., and Shimojo, S. (2000). Illusions–what you see is what you hear. *Nature.* 408, 788–788. doi: 10.1038/35048669

Spence, C. (2011). Crossmodal correspondences: a tutorial review. *Atten. Percept. Psychophys.* 73, 971–995. doi: 10.3758/s13414-010-0073-7

Stafstrom, J. A., and Hebets, E. A. (2013). Female mate choice for multimodal courtship and the importance of the signaling background for selection on male ornamentation. *Curr. Zool.* 59, 200–209. doi: 10.1093/czoolo/59.2.200

Stange, N., Page, R. A., Ryan, M. J., and Taylor, R. C. (2017). Interactions between complex multisensory signal components result in unexpected mate choice responses. *Anim. Behav.* 134, 239–247. doi: 10.1016/j.anbehav.2016.07.005

Starnberger, I., Preininger, D., and Hoedl, W. (2014). From uni- to multimodality: towards an integrative view on anuran communication. *J. Comp. Physiol. A.* 200, 777–787. doi: 10.1007/s00359-014-0923-1

Stein, B. E. (2012). *The New Handbook of Multisensory Processing.* Cambridge, MA: MIT Press.

Stein, B. E., Stanford, T. R., and Rowland, B. A. (2014). Development of multisensory integration from the perspective of the individual neuron. *Nat. Rev. Neurosci.* 15, 520–535. doi: 10.1038/nrn3742

Taylor, R. C., and Ryan, M. J. (2013). Interactions of multisensory components perceptually rescue Tungara frog mating signals. *Science.* 341, 273–274. doi: 10.1126/science.1237113

Uetz, G. W., and Roberts, J. A. (2002). Multisensory cues and multimodal communication in spiders: Insights from video/audio playback studies. *Brain Behav. Evol.* 59, 222–230. doi: 10.1159/000064909

Ullrich, R., Norton, P., and Scharff, C. (2016). Waltzing *Taeniopygia*: integration of courtship song and dance in the domesticated Australian zebra finch. *Anim. Behav.* 112, 285–300. doi: 10.1016/j.anbehav.2015.11.012

Vedenina, V. Y., and Shestakov, L. S. (2018). Loser in fight but winner in love: how does inter-male competition determine the pattern and outcome of courtship in cricket *Gryllus bimaculatus*? *Front. Ecol. Evol.* 6:197. doi: 10.3389/fevo.2018.00197

Voigt, C. C., Behr, O., Caspers, B., von Helversen, O., Knornschild, M., Mayer, F., et al. (2008). Songs, scents, and senses: sexual selection in the greater sac-winged bat, *Saccopteryx bilineata*. *J. Mammal.* 89, 1401–1410. doi: 10.1644/08-mamm-s-060.1

Whitchurch, E. A., and Takahashi, T. T. (2006). Combined auditory and visual stimuli facilitate head saccades in the barn owl (*Tyto alba*). *J. Neurophysiol.* 96, 730–745. doi: 10.1152/jn.00072.2006

Williams, H. (2001). Choreography of song, dance and beak movements in the zebra finch (*Taeniopygia guttata*). *J. Exp. Biol.* 204, 3497–3506.

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REVIEW
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Multimodal Signaling in Myrmecophilous Butterflies

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Ant nests and their surrounding territories represent a hoard of trophic resources, as well as of stable and protected environments for many arthropods involved in commensal, mutualistic, or parasitic associations. Among these organisms, called myrmecophiles, several are butterflies. Here, we explore the amazing diversity of strategies developed by myrmecophilous butterflies to "cheat" or manipulate ants and to elude the tough defenses of the colony. During oviposition, female butterflies use visual or plant volatile signals to identify the presence of ants, whereas chemical and vibroacoustic cues, either isolated or combined, are used by larvae and pupae to attract, deceive, or appease workers. Examples of mimicry and eavesdropping on both intraspecific and interspecific signals are discussed, primarily referring to the obligate-parasitic interactions involving *Maculinea* butterflies and *Myrmica* ants. Multimodal communication is crucial to maintaining the strong cohesion and social structure of ant societies, but its corruption is at the base of the evolution and persistence of interspecific associations, which can be beneficial or detrimental for the colony's fitness. In this framework, the remarkable complexity of signaling could have prompted the evolution of specialized life cycles enhancing the extraordinary butterfly diversity.

Keywords: ants, chemical cues, eavesdropping, manipulation, mimicry, oviposition, vibroacoustic signals

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INTRODUCTION AND OUTLINES

Peculiar attributes of ants such as their ecological dominance, wide distribution, and eusocial organization led to the evolution of a variety of associations sometimes with distantly-related organisms, called myrmecophiles (ant-loving) (Hölldobler and Wilson, 1990).

Myrmecophilous organisms tend to be exceptionally cryptic and spend variable amounts of their lives within insect societies. Therefore, the occurrence of myrmecophily across taxa (e.g., Hymenoptera, Diptera, Coleoptera, and Lepidoptera) could have been deeply underestimated. Nevertheless, based on Donisthorpe's work (Donisthorpe, 1927) and several other studies, Thomas et al. (2005) estimated that around 100,000 myrmecophilous species of invertebrates exist.

Among the others, the diversity, functional and evolutionary ecology of butterfly-ant interactions have been extensively reviewed by Hinton (1951), Malicky (1969), DeVries (1991b), Fiedler (1991, 1994), and Pierce et al. (2002). However, little attention has been paid to the signaling system necessary to foster and maintain myrmecophilous associations, even though interspecific communication is vital for these relationships.

By means of food secretion or furtiveness, the majority of myrmecophiles (commensals or mutualists) live undisturbed or actively protected within the territories of ants, but others are specialized to overcome the colony defenses and succeed in entering the ant nests (Hölldobler and Wilson, 1990; Thomas et al., 2005). Some myrmecophiles are obligately associated with ants and have evolved remarkable strategies "to break the communication code" of their symbionts (Hinton, 1951; Schönrogge et al., 2017). Being able to exploit the fiercely defended ant territory or the nest itself by "cheating" ants would provide the myrmecophilous organisms with several advantages [e.g., protection against predator and parasites, shelter, transport, and a potential depletion of pathogen contamination (New, 2017)] as well as a persistent source of food and protection (Wasmann, 1913; Barbero, 2016).

DeVries (1991c) suggested that butterfly-ant associations could have arisen as a consequence of earlier and long-lasting interactions between ants and angiosperms (Moreau et al., 2006). Ants can exploit plants or their litter as nesting resources (Wilson and Hölldobler, 2005) but can also prey on or benefit of the secretions elicited by plants or plant-associated herbivores (Davidson et al., 2003). To take advantages of ant protection against their natural enemies, plants have evolved amazing adaptations to attract them directly via extrafloral nectar exudations or indirectly by means of honey-dew secreting hemipterans (Rico-Gray and Oliveira, 2007). On the other hand, butterflies, that primarily need plants as food for their larval offspring, also started to appease patrolling ants to obtain protection against predators or parasitoids in the most vulnerable period of their life (i.e., larval development and pupation). This results in a faster growth rate and a higher survival or better reproductive success of attended individuals with respect to unattended ones (Cottrell, 1984; Pierce et al., 1987; Kaminski et al., 2013).

The larvae and pupae of myrmecophilous species lack a broad array of protections typical of other butterflies, such as defensive secretions, dense "hairiness," or sequestration of toxic plant metabolites and associated aposematic colorations (Fiedler, 1991). However, in addition to food rewards by specialized organs, butterflies have developed an array of morpho-physiological, behavioral, chemical, or acoustic adaptations to attract, trick, alarm, or pacify their associated ants, which is discussed here.

Our review is narrowed to the exchange, emission, perception, manipulation, and eavesdropping of signals occurring in associations involving myrmecophilous butterflies because these systems (i) have been studied by experts of distinct disciplines providing insights on their function and biology; (ii) are textbook models of coevolutionary dynamics; (iii) often involve other trophic levels (e.g., plants and parasitoids) thereby providing the rare opportunity to explore the complexity of the "information web" interlaced with the "food web" (Dicke, 2000).

Given that myrmecophily is mostly present in two butterfly families (Lycaenidae and Riodinidae), which encompass about 30% of all butterfly species (Shields, 1989) most of the studies were performed on these taxa. Within these families, 75% of the species possess juvenile instars interacting with ants at various stages of the life cycle showing a variety of associations ranging from facultative to obligate and from mutualistic to parasitic (Pierce et al., 2002). For those lycaenid species whose life history is fully known, about 30% are closely associated with ants (obligate myrmecophiles), 45% are facultative myrmecophiles and about 25% show no association with ants (Pierce et al., 2002).

Depending on their degree of interaction, which summarizes time, space, kind, and specificity of the associations (Hinton, 1951), myrmecophilous adult butterflies can (i) use direct and indirect signals to detect the presence of ants in order to select ideal oviposition sites (see Signals Used During Butterfly Oviposition); while immature stages can (ii) secrete pacifying, rewarding or manipulating substances by dorsal nectary organs (DNOs) to gain protection against predators (see Chemical Signals); (iii) release allomones or alarming volatile compounds from tentacle organs (TOs) or other specialized organs to get access to their food sources (see Chemical Signals); (iv) subvert the nestmate recognition system based on cuticular hydrocarbons (CHCs) to enter and live in the ant nest (see Chemical Signals); and (v) produce mimetic vibroacoustic signals to attract the attention of ants (see Vibroacoustic Signals).

After introducing signal systems occurring in facultative and unspecialized interactions, we focus our review on obligate parasitic associations. In extreme cases, such as in the parasitic *Maculinea* butterflies, the interaction is so tight and close that requires the corruption of several host signals (both chemical and acoustical) for the butterfly juveniles to be accepted and treated like colony members by the host ants. These butterflies show a peculiar life cycle (Thomas, 1984) (**Figure 1**), which will be described throughout this review, highlighting the multimodal signaling which makes this parasitism successful. In brief, after egg-laying on species-specific food plants occurs, larvae spend a short phytophagous period gaining little body mass. Then caterpillars leave the flower buds and wait motionless before being discovered by a *Myrmica* ant. Following an adoption ritual of variable duration depending on the species, during which behavioral (Fiedler, 1990), chemical (Akino et al., 1999; Nash et al., 2008), and vibroacoustic (Sala et al., 2014) signals are used by the parasite in order to pretend to be a *Myrmica* larva, the "cheated" forager ant carries the parasite within its nest. Once inside the colony, *Maculinea* caterpillars integrate into the host colony by using chemical and vibroacoustic deceiving signals (Schönrogge et al., 2004; Barbero et al., 2009b). Some species directly prey on the ant brood ("predatory" species), while others ("cuckoo" species) are fed by food regurgitation by the nurse workers. In the nest, the final larval body mass is achieved and pupation occurs in the upper nest chambers. Thus, after 11–23 months -as 2-year developing larvae exist within the same population to cope with catastrophic events or habitat unpredictability (Thomas et al., 1998; Witek et al., 2006)- *Maculinea* butterflies leave the ant nest as adults.

Primarily using the case of *Maculinea* butterflies, we endeavor to highlight the importance of surveying the signaling beyond the pairwise level of organism interactions, by providing evidence that eavesdropping might occur when multiple connections (ant, butterfly, plant, parasitoids) are considered. Communication and its corruption are at the base of the evolution and maintenance of multiple interactions, and we believe that community context approaches are ideal to assess properly all the selective pressures shaping these complex systems. We discuss the hypothesis by

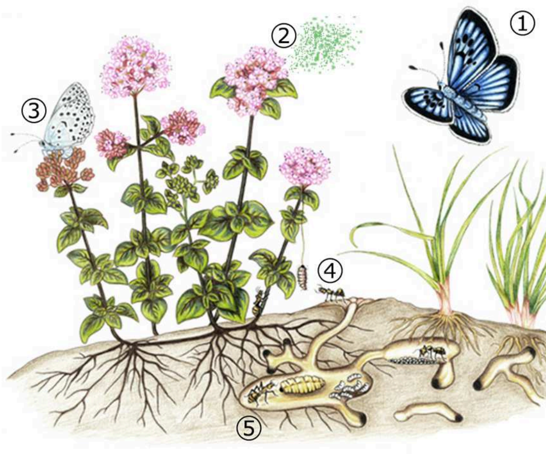

FIGURE 1 | Life cycle of *Maculinea arion* and its interaction with *Myrmica* ants and *Origanum vulgare* following Patricelli et al. (2015). (1) Gravid female butterflies are visually attracted to the green-red buds of *O. vulgare*; when in their proximity, (2) by detecting the monoterpenoid volatile carvacrol emitted by plants co-occurring with ants of any *Myrmica* species (3) female lays eggs on phenologically suitable flower buds; (4) fourth-instar caterpillars leave the host plant and are "adopted" by *Myrmica* ants; (5) *M. arion* caterpillars spend 11 months within *Myrmica* colonies, feeding on ant brood and acquiring more than 98% of their final biomass. Artwork by Elisa Plazio.

several authors (Malicky, 1969; Atsatt, 1981; DeVries, 1991b,c; Fiedler, 1998; Pierce et al., 2002; Stadler et al., 2003; Pech et al., 2004; Pellissier et al., 2012; Schär et al., 2018) that the interactions with ants represented one of the most important factors in the butterfly (Lycaenidae) adaptive radiation.

SIGNALS USED DURING BUTTERFLY OVIPOSITION

Female choice of an ideal egg-laying site is fundamental for offspring survival and consequently for the persistence of butterfly populations (Chew and Robbins, 1984; Renwick and Chew, 1994). The observed egg distribution on larval host plants (LHPs) is the outcome of several biotic and abiotic factors, such as the plant species, quality or distribution, larval intraspecific competition, microclimatic conditions, presence of mutualists or predators (Chew and Robbins, 1984; Renwick and Chew, 1994). Since early records of myrmecophilous behavior in butterflies, several authors have argued that selection may have favored the ability to locate both the LHPs and the host ants (Pierce and Elgar, 1985; Fiedler and Maschwitz, 1989a,b; Jordano et al., 1992; Wagner and Kurina, 1997; Patricelli et al., 2011). Laying eggs in the proximity of ant colonies would increase the probability of the juveniles to encounter the tending workers and this achievement is even more crucial in strictly obligate myrmecophilous species (Van Dyck et al., 2000). Hence it has been hypothesized that ants themselves, the release of their pheromones or trail compounds can serve as mating and oviposition cues.

The first empirical evidence of ant-mediated oviposition was gathered through field studies assessing lycaenid egg distribution on food plants with respect to ant presence/absence. Pierce and Elgar (1985) reported that females of the obligate mutualist species, *Jalmenus evagoras*, laid their eggs primarily on food plants colonized by honeydew-producing aphids to maximize chances of their larvae to be visited by the *Iridomyrmex* host ants. However, *J. evagoras* females did not perceive the workers directly, but followed indirect visual cues (i.e., the aphid occurrence) as proxies for the ant presence (Pierce and Elgar, 1985). In this species, males use the presence of ants and conspecific adults as mating signals also to spot newly-emerged females (Elgar and Pierce, 1988). Further field and laboratory experiments revealed that *J. evagoras* females are able to discriminate between species and to some extent also populations of attendant ants and lay their eggs close to their dominant and syntopic ant species (Fraser et al., 2002). Beyond suggesting that oviposition preferences could have a genetic basis, these results recall the possibility that "a behavioral imprinting process" where the ability to perceive chemical and visuals signals produced by the associated ants is established during butterfly development (Fraser et al., 2002). Yet, the direct perception of ant chemicals by gravid females remains to be formally tested.

Despite earlier insights (Pierce et al., 2002), recent works suggest the occurrence of ant-related oviposition choices also in facultative associations (Rodrigues et al., 2010; Bächtold et al., 2014). Most eggs and larvae of the facultative lycaenid, *Allosmaitia strophius*, were laid on stems of *Peixotoa tomentosa* an extrafloral nectaried shrub where *Camponotus blandus* and *Ectatomma tuberculatum* ants occur (Bächtold et al., 2014). Unfortunately, authors did not perform an in-depth survey of the signals used by gravid females to select plants visited by ants, which are therefore unknown. We cannot rule out that only visual cues are employed as they can be exceptionally precise, like it was demonstrated in the non-myrmecophilous *Eunica bechina*, a nymphalid butterfly which lays eggs on an ant-defended plant. In this case, however, females are able to discriminate the shape of more aggressive ants by sight thus preventing the oviposition on deadly ant-patrolled plants (Sendoya et al., 2009).

Although a strong ant-driven oviposition behavior is expected to maximize offspring survival in the obligate parasites of the genus *Maculinea*, which are locally hosted by one or few ant species and exploit rare and specific LHPs (Thomas et al., 1989), contrasting results are found for distinct populations. While field data consistently support the female ability to select specific LHP parts, characteristics, and bud blooming stages, some studies pointed out that females select LHPs primarily on the basis of the plant phenology rather than on the local host ant presence (Thomas and Elmes, 2001; Musche et al., 2006; Fürst and Nash, 2009; Czekes et al., 2014). Others revealed a role of the host ant distribution on the egg-laying pattern (Van Dyck et al., 2000; Wynhoff et al., 2008), but this outcome has been explained by Thomas and Elmes (2001) as the consequence of a food plant niche selection rather than a direct ant-driven oviposition choice. Wynhoff et al. (2015) found that the selection of the LHP by *Maculinea alcon* is not influenced by the host ant presence, but the number of eggs laid (egg load) increased on plants growing close to a *Myrmica* nest. In *M. rebeli,* a *M. alcon* close-related species, both the egg-laying probability and the egg loads on *Gentiana asclepiadea* are affected by the abundance of the host ant, *M. scabrinodis* (Carleial et al., 2018). The oviposition pattern observed is not a mere consequence of the positive effect of plant traits, such as flower numbers (also selected by the gravid females for egg laying), on *Myrmica scabrinodis* distribution. Authors highlighted that both the plant features and the host ant presence have direct, distinct, and positive effects on oviposition choices, which cannot be considered as a spurious outcome of niche selection (Carleial et al., 2018). The majority of earlier studies on oviposition choices did not account for the contribution of all *Myrmica* species occurring in the surrounding of LHPs but focused on the local host ant distribution. In contrast, Patricelli et al. (2011) found a strong correlation between *Maculinea arion* egg-laying preferences and the occurrence of any (not necessarily the local host) *Myrmica* species around the LHPs chosen. Approximately 80% of egg-laying events occurred on LHPs surrounded by *Myrmica* ants, suggesting the existence of a mechanisms by which females are able to identify plants growing in the proximity of a *Myrmica* colony.

An ant-mediated oviposition behavior is not so straightforward in all *Maculinea* species or populations, whereas plant features are crucial. Visual cues can be employed to spot the plant phenology and after landing females might avail themselves of the chemical cues related to the ant presence to decide how many eggs are worth laying. Although in other myrmecophilous butterflies the ability to directly detect the presence of ants by chemical or visual cues have been inferred or shown (respectively), this seems unlikely to occur in the *Maculinea-Myrmica* system (Thomas and Elmes, 2001) because: (i) *Myrmica* ants usually forage when female butterflies are less active in laying eggs, (ii) workers release highly volatile pheromone trails which fade quickly thus making it difficult for *Maculinea* females to follow them, and (iii) females do not frequently exhibit complex searching behavior (but see Van Dyck et al., 2000).

Females possess the full array of organs and receptors on antennae, tarsi, mouthparts or ovipositor to enable a precise detection of several chemical signals in order to select the right LHPs and to assess their quality. Therefore, some authors started to hypothesize that induced plant volatiles might work as indirect signals for the ant presence and provide hints for the egg-laying females (Van Dyck and Regniers, 2010; Wynhoff et al., 2015). Patricelli et al. (2015) provided the first evidence that *Origanum vulgare* plants, the LHP of *M. arion*, react to *Myrmica* workers by releasing a volatile organic compound (carvacrol), which in turn is used by gravid females to locate ideal food plants growing close to a *Myrmica* nest (**Figure 1**). Experimental *O. vulgare* plants grown with ants upregulate genes involved in the monoterpenes pathway thus releasing higher amounts of carvacrol than control plants (without ants). This monoterpene is perceived by butterfly antennae and attracts the gravid females in laboratory choice tests. Carvacrol has detrimental effects on ants, but by the upregulations of detoxifying genes these workers survive longer than other common ant species to environmental concentrations of this monoterpene compound. Therefore, authors initially suggested that *Myrmica* ants benefit from being resistant to carvacrol by occupying enemy-free spaces close to *O. vulgare* plants at the cost of enhancing their chances of being parasitized by *M. arion*. Very recently, it has been shown that this ant-plant interaction could be maintained through manipulation signals. Mixtures of carvacrol and thymol decrease *Myrmica* ant movements and increase their aggressive behaviors acting on the brain levels of biogenic amines (Mannino et al., 2018). Therefore, similarly to the scenario described by Hojo et al. (2015) (for further details see below), here oregano seems to manipulate *Myrmica* ants by volatile cues in order to increase partner fidelity and obtain protection against herbivores by patrolling workers.

Using a multidisciplinary approach, authors shed light on the indirect mechanism used by *M. arion* females to detect the presence of the *Myrmica* ants by means of a very tiny variation in the LHP volatilome (Patricelli et al., 2015) and on the way a certain degree of overlap between the two butterfly resources, LHPs and *Myrmica* ants, can be maintained (Mannino et al., 2018).

During the revision of papers dealing with oviposition behavior in myrmecophilous butterflies we did not find any robust evidence of the fact that females are able to directly detect the ant presence by perceiving their chemical cues. Indeed, this is only partially surprising because (i) butterflies coevolved primarily with their host plants, achieving remarkable abilities in sensing their signal variation as proxies for plant features and phenological states; and (ii) a chemical reaction is the most probable response of a plant which is interacting with insects, including ants. Therefore, it is likely that among the multifarious plant signals are those exploited by females to indirectly gain information about their LHP degree of association with ants.

CHEMICAL SIGNALS

The interaction between immature butterfly stages and ants is mediated above all by different mechanisms that involve the production of chemical substances.

Nectary Organs

In several species belonging to the subfamily Lycaenidae and Riodinidae one of the main mechanisms that allow larvae to manipulate ant behaviors by attracting and maintaining the attention of workers is the production of rewarding exudations. In lycaenids these secretions are released from the dorsal nectar organ (**Figure 2B**) (DNO; Newcomer, 1912; Fiedler and Maschwitz, 1989c; Leimar and Axén, 1993; Pierce et al., 2002) located on the seventh abdominal segment and in riodinids from paired tentacle nectary organs (TNOs) located on the eighth abdominal segment (DeVries, 1988).

In Lycaenidae, many factors influence the production of rewarding secretions made available to mutualistic ants (Agrawal and Fordyce, 2000). When exposed to simulated threats, caterpillars of both *Polyommatus icarus* (Leimar and Axén, 1993) and *Plebejus acmon* (Agrawal and Fordyce, 2000) secrete more rewards and attract a higher number of attendant ants. However, beyond a threshold number of attendant ants, the benefit from producing metabolically expensive secretions may have scarce

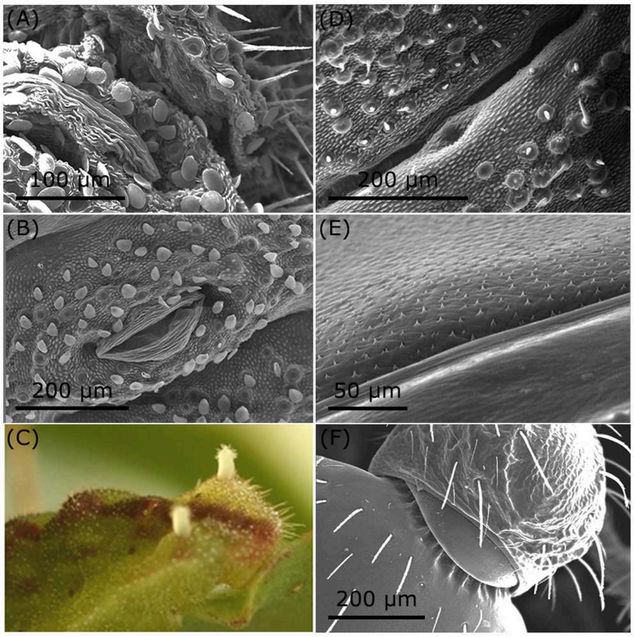

FIGURE 2 | Morphology of organs producing chemicals and vibroacoustic signals in myrmecophiles and their host ants. **(A)** Pore cupola organs (PCOs) and **(B)** dorsal nectary organ (DNO) of fourth-instar *Maculinea rebeli* caterpillars; **(C)** tentacular organs of fourth-stage *Scolitantides orion* caterpillar; **(D)** the possible sound-producing organ of *M. rebeli* caterpillars; **(E)** stridulatory organ of *M. rebeli* pupa, formed by a stridulatory plate (*pars stridens*) located on the sixth abdominal segment and a file (*plectrum*) in the fifth abdominal segment. **(F)** Stridulatory organ of *Myrmica sabuleti*.

returns (Leimar and Axén, 1993; Axén et al., 1996). The rate at which larvae provide rewards may also depend on social context. Aggregated caterpillars of *Jalmenus evagoras* deliver less food rewards to ants than solitary larvae, and secretion rate decreases with increasing group size. Also, secretion rates are lower when IV stage caterpillars are paired with bigger V instar larva than when aggregate to a smaller III instar individual (Axén and Pierce, 1998).

In general, carbohydrates and amino acids represent the main components of these secretions, whose composition is unlikely to be explained by caterpillar diet (DeVries, 1988) or by the contents of the hemolymph but is more likely to be genetically determined and related to the degree of association with ants (Pierce et al., 1987; Daniels et al., 2005). In all species of Lycaenidae and Riodinidae analyzed so far, the secretions contain different combinations of sugars, one of them being usually more abundant. Sugars are principally sucrose and glucose (Daniels et al., 2005) in dilutions of around 5–10%, except for the Australian species *Paralucia aurifera* where glucose, the only carbohydrate, reaches average concentrations of 34% (Cushman et al., 1994). Secretions of the parasitic butterfly *Niphanda fusca*, contain three types of sugars of which trehalose shows the greatest concentrations (380 mmol 1-1) (Hojo et al., 2009), while in the obligate mutualist *Jalmenus evagoras* secretions consist of about 10% (dry weight) of sucrose and fructose (Pierce and Nash, 1999). The latter were the main components also in the droplets of the facultative mutualists *Polyommatus hispanus* (Maschwitz et al., 1975), *Polyommatus coridon*, *P. icarus*, and *Zizeeria knysna* (Daniels et al., 2005).

Also the amino acid content of the dorsal nectar, whose concentration is slightly higher than in aphid honeydew (Yao and Akimoto, 2002) and in floral and extrafloral nectars (Blüthgen et al., 2004), seems to be determined by the intimacy of associations with ant attendants, since a richer and more diversified mixture is likely to be produced by more strongly myrmecophilous species (Pierce et al., 1987; Daniels et al., 2005). For instance, the amino acid content of *P. coridon* secretions, one of the most strongly ant-associated, among facultative myrmecophilous species, reaches the highest concentrations (108 mmol l-1) among the caterpillars analyzed so far, with leucine being the predominant constituent (Daniels et al., 2005). In contrast, *P. icarus* and *Z. knysna*, which are weakly myrmecophilous, have low amino acid levels (respectively, 10 and 6 mmol l^{-1}).

Several authors have demonstrated that the quantity and quality of nutritive rewards influence the persistence of guarding ants in attending caterpillars (Pierce et al., 2002). DNO secretions of the parasitic caterpillars of *Niphanda fusca* contain high concentrations of the amino acid glycine associated to several carbohydrates, i.e., mostly trehalose (Hojo et al., 2009) and glucose (Wada et al., 2001). Electrophysiological recordings of the taste receptors of the host ant species, *C. japonicus*, showed that the presence of even small amounts of glycine, combined with the main sugars, made attendant ants more attracted to these solutions and enhanced the electrophysiological response to sugar of sugar-receptor cells (Wada et al., 2001; Hojo et al., 2009).

Recent observations (Hojo et al., 2015) suggest that DNO droplets may be more than simply nutritious recompenses. In a supposed mutualistic lycaenid, *Narathura japonica,* DNO secretions, or CHCs (see below) alone do not elicit ant-caterpillar interaction, but together they act synergistically to promote ant allegiance (Hojo et al., 2014). DNO caterpillar secretions lower the locomotory activities of their attendant *Pristomyrmex punctatus* ants (Hojo et al., 2015) and increase the frequency of aggressive responses to tentacle organ eversion. Analysis of the neurogenic amines in the brains of ants that consumed caterpillar secretions showed a significant decrease in levels of dopamine, suggesting that DNO secretions of lycaenid larvae can manipulate attendant ant behavior by altering dopaminergic regulation and increasing partner fidelity (Hojo et al., 2015). Because a net nutritional benefit from the DNO droplets is doubtful, authors suggested this interaction which has been traditionally considered a mutualism could be indeed a parasitic association.

In parasitic lycaenid butterflies possessing in some cases large functional DNOs (Samson, 1989; Nomura, 1992; Sanetra and Fiedler, 1996), it is likely that ant manipulation is achieved solely by using other chemical (and vibroacoustic) strategies, e.g., chemical mimicry of CHCs (see below). For instance, final larval instars of *Maculinea rebeli* spend on average 18% of time actively secreting DNO droplets during the 11–23 months spent within *Myrmica* colonies (Elmes et al., 1991). Yet, they do not recycle sufficient sugar through their secretions to provide significant rewards to the adult ants, which in turn experience higher mortality and lower fitness when the colony is parasitized by *M. rebeli* (Wardlaw et al., 2000).

Tentacle Organs

The larvae of many myrmecophilous Lycaenidae and Riodinidae have a second pair of extensible organs to interact with ants. In lycaenids these organs, called "tentacle organs" -TO- (**Figure 2C**) (Cottrell, 1984; Kitching and Luke, 1985), are located on the eighth abdominal segment while a pair of "anterior tentacle organs" -ATO- (DeVries, 1988) has been detected on the third body segment of caterpillars of all myrmecophilous riodinids, except for the genus *Eurybia* (Harvey, 1987; DeVries, 1991c).

Although the function of tentacle organs has been debated over several decades, it has not been fully clarified yet. Some authors suggested that TOs may induce a response in ants by functioning as a tactile or visual cue (Murray, 1935; Malicky, 1969). This hypothesis may be partially corroborated by a recent work by Gnatzy et al. (2017) that investigated the internal fine structure of the TOs of *P. coridon* and *P. icarus* larvae and failed to find evident glandular structure or sign of secretory activity.

However, several authors have reported that the eversion of the TOs induce alertness or even alarm behavior in the attendant ants (Claassens and Dickson, 1977; Fiedler and Maschwitz, 1988, 1989c). Ants' response is usually observed only at a close range from the tentacle organs and not all ant species exhibit a reaction when a certain species of lycaenid extrudes its organs. The similarity of behavior of an attendant ant to ant reaction during a threatening situation, the specificity of the observed reaction, and the short range of activity led several authors (Henning, 1983; DeVries, 1984; Kitching and Luke, 1985) to suggest that TOs could produce volatile compounds mimicking ant alarm pheromones.

Support for this pheromone-mimic hypothesis initially came from a study by Henning (1983) who was able to obtain a dichloromethane extract of the TOs and the surrounding body area of the lycaenid *Aloeides dentatis*. In behavioral assays, alarming responses obtained from *Acantholepis capensis* attendant ants exposed to these extracts were similar to those of ants presented with dichloromethane extracts from conspecific mandibular glands (Henning, 1983).

A few years later, the secretions from the tentacle organ of another lycaenid species, *Shirozua jonasi,* were shown to contain dendrolasin, a C15 furan sequiterpenoid (Yamagushi and Shirozu, 1988), firstly isolated from the mandible glands of the ant *Lasius fuliginosus* where it may function as an alarm pheromone (Quilico et al., 1957). However, the chemicals released from the TOs and ATOs remain largely unknown (Pierce and Nash, 1999).

Pore Cupola Organs

On the cuticle of all lycaenid and riodinid immature stages, except for the myrmecophage *Liphyra brassolis* (Fiedler, 1991), a third set of minute epidermal glandular structures called pore cupola organs -PCOs- (Cottrell, 1984) are present (**Figure 2A**). Their morphology and distribution differ markedly between taxa (Kitching, 1987), but PCOs are generally present starting from the first instar and their density increases at every molt (Malicky, 1969). Also, in most lycaenid species PCOs concentrate around the spiracles and (if present) around the DNO (Fiedler, 1991).

Some caterpillars can be visited by several ants (Pierce, 1984) and caterpillars equipped with PCOs can elicit antennae behavior at different intensities. Some species arouse little attraction in ants, while others receive constant antennation (Malicky, 1969; Fiedler, 1991). During ant-caterpillar interactions, the densest PCO areas are frequently antennated and groomed by ants, suggesting that they may secrete ant-appeasing substances. In other cases, the signals have been considered as pacification cues (Maschwitz et al., 1985). PCOs can secrete amino acids in some species (Pierce, 1984). Malicky (1969) suggested that these chemical signals suppress ant aggression by mimicking ant chemical cues and specifically those emitted by the colony brood. More specifically, given that ant interest arises only after the first contact, it has been suggested that PCOs may be responsible for the production of CHCs, non-volatile compounds (Pierce, 1984) which are known for signaling colony membership, in ant societies.

However, whether PCOs are involved in the production of CHCs remains to be confirmed. Especially because, while PCOs are unique to lycaenid caterpillars, CHCs are universal among insects being primarily important in preventing desiccation (Gibbs, 1998) and facilitating osmoregulation (Nelson and Blomquist, 1995).

Cuticular Hydrocarbons

It is well-known that various myrmecophiles including butterflies exhibit CHCs that mimic those of their ant hosts to avoid ant attacks (for butterflies, Akino et al., 1999; Hojo et al., 2009; Barbero, 2016; Mizuno et al., 2018). In general, CHCs play a fundamental role in the nestmate recognition of social insects, and in particular of ants (van Zweden and d'Ettorre, 2010). Ants living in the same colony share a mixture of chemicals, which function as a "colony odor" and enables them to discriminate between nestmates and enemies (Lenoir et al., 2001).

Therefore, irrespective of their intensity of interaction, acquiring a composition of CHCs that are recognized and accepted by the attending ants is the most common, and almost essential, strategy for myrmecophilous butterfly caterpillars to exploit ant societies (Barbero, 2016 and references therein).

Hojo et al. (2014) demonstrated that CHCs are used by the mutualistic lycaenid *N. japonica* to be recognized by its tending ants *P. punctatus*. Indeed, glass beads coated with crude cuticular chemicals of *N. japonica* and CHC fractions extracted from caterpillars were significantly more tended by *P. punctatus* ants than control glass dummies or dummies coated with the non-hydrocarbon fraction. A comparison of caterpillar cuticle extracts of *N. japonica* and the myrmecoxenous *Lycaena phlaeas* revealed that the total amount of CHCs were not significantly different but differed in composition. While caterpillars of *L. phlaeas* have a simple set of hydrocarbons, mainly *n*-alkanes, *N. japonica* caterpillars have a complex mixture of *n*-alkanes, *n*-alkenes, and *n*-alkadienes, so that it is likely that the host ant would selectively recognize the unsaturated hydrocarbon fraction and use it for its nestmate recognition system (Hojo et al., 2014).

In another facultative myrmecophilous butterfly, *Lycaeides argyrognomon*, the CHC profiles of larvae and pupae are almost identical (a few methyl-branched alkanes lacking in the pupae), but differ from those of any host ant species (Omura et al., 2009). Unlike parasitic lycaenid larvae (see below), mutualistic lycaenids probably have not evolved CHC profiles mimicking ant CHCs of a particular species (Omura et al., 2009). The CHC profiles of *L. argyrognomon* larvae and pupae are dominated by *n*-alkanes, which may contribute to the lack of ant aggression (Dani et al., 2001; Omura et al., 2009). It is likely that in mutualistic species, associative learning of chemicals and DNO reward secretion can be accomplished with one or a few compounds (Guerrieri et al., 2009). Indeed, workers of several ant species can learn a specific blend of hydrocarbons when provided with nectar solutions (Bos et al., 2012; Hojo et al., 2014). In addition, in mutualistic butterflies other mechanism of ant manipulation may exist. Mizuno et al. (2018) found that cuticular lipids of pupae do not include only CHCs but also several long-chained aliphatic aldehydes, including 1-octacosanal and 1-triacontanal, which are absent from the larva and are responsible for suppressing ant aggression in certain attending ants during the pupal stage (Mizuno et al., 2018).

However, it is straightforward that the need to possess specific chemical adaptations to bypass the chemical recognition barriers is essential in obligate myrmecophiles, which enter the ant colonies to exploit their resources (Singer, 1998; Barbero, 2016). Obligate caterpillars imitate the CHC profile of their ant hosts to be adopted as nestmates and thereby integrate into the colonies of their hosts (Akino et al., 1999; Hojo et al., 2009). Henning (1983) was first to demonstrate that caterpillars of the inquiline species *Lepidochrysops ignota* possess cuticular compounds inducing carrying and brood-caring behavior in the specific host ant, *Camponotus niveosetosus*. Interestingly, post adoption caterpillars of the parasitic lycaenid butterfly *Niphanda fusca* exploit worker care by imitating the CHC profiles of host ant males rather than of the ant larvae of their host *Camponotus japonicus* (Hojo et al., 2009). Since the parasitic caterpillars are principally attended by workers and do not actively contact the host males, it is possible that *N. fusca* larvae are able to biosynthesize the mimetic CHCs that serve for integrating into the host colony (Hojo et al., 2009).

Probably the most intensively studied system from a chemical point of view is that of the parasitic species belonging to the genus *Maculinea*, whose strategies of chemical deception will be treated in detail.

Elmes et al. (1991) initially suggested that the system by which the butterflies of the genus *Maculinea* could enter the colonies of *Myrmica* ants was mediated by the imitation of host chemical signals. This hypothesis was confirmed by Akino et al. (1999) for the cuckoo species, *Maculinea rebeli*. In behavioral tests, workers of the host ant *Myrmica schencki* carried into their nest glass dummies covered with cuticular extracts of fourth-instar caterpillars.

The comparison between the CHCs of *M. rebeli* caterpillars and *M. schencki* workers confirmed that during the pre-adoption phase the parasitic caterpillars possess a CHC profile that weakly mimics that of its main host species (Akino et al., 1999; Elmes et al., 2002; Schönrogge et al., 2004). Compared to its host ants which usually have complex CHC profiles, the *M. rebeli* pre-adoption caterpillars have only a dozen of CHC compounds, most of which are linear alkanes, while methylated alkanes usually constitute no more than 5% of the total amount of linear compounds (Akino et al., 1999; Elmes et al., 2002; Schönrogge et al., 2004).

The CHC cuticular fraction seems to contain chemical cues which can be recognized by any *Myrmica* ant encountering a fourth-instar larva leaving its food plant. Nevertheless, the matching of host surface hydrocarbons can largely influence the adoption time and explain the differences in host use observed in *Maculinea* populations (Nash et al., 2008). For instance, caterpillars of the other cuckoo species, *M. alcon,* from Denmark are adopted an order of magnitude faster by colonies of *M. rubra* or *M. ruginodis*, which are suitable as host colonies, than by colonies of non-host *M. scabrinodis* (Als et al., 2001).

In a recent study where the chemical profiles of *M. rebeli* pre-adoption larvae were compared to those of *Myrmica* workers sampled in Italy at six locations, authors found a direct relationship between the chemical similarity of caterpillars and host workers, and the estimated survival rate of the social parasite within the ant colonies where more than one host species is used. The chemical similarity between pre-adoption *M. rebeli* larvae and *Myrmica* ants explained a significant proportion (around 30%) of the variation in the estimated survival of parasitic caterpillars (Casacci et al., 2019). Similar results were found by Thomas et al. (2013) who demonstrated that differences in the CHC profiles of two *M. rebeli* populations from Spain and Poland are strong enough to explain the differences in the local host ant use.

Additionally, it is likely for the adoption process to be mediated by few active compounds as shown by Solazzo et al. (2014). Indeed, through behavioral assays and chemical analyses, the authors proposed that tetracosane, a low volatility compound present on *M. nausithous* cuticle in the pre-adoption phase, plays a role in this process by enhancing the first interaction with *M. rubra* foraging workers.

After 1 week within the host colony, the CHC profile of *M. rebeli* caterpillars change and larvae become chemically closer (more than 60% similarity) to their hosts (Schönrogge et al., 2004). Initially, it was hypothesized that the complete integration of the parasitic larvae depended on the passive absorption of colony odors through contact and exchange of secretions with the workers know as "chemical camouflage" (*sensu* Dettner and Liepert, 1994). Subsequently, however, it was highlighted that individuals that overcame the period of "initial integration" survived well with non-host species of *Myrmica*, only as long as the colony remained well-nourished and in favorable conditions. If, however, as often happens in nature, the colony was undergoing a lack of trophic resources or other types of stress, the larvae of *M. rebeli* continued to survive well with the local host *Myrmica schencki*, while caterpillars in *Myrmica* "non-host" colonies were killed and used as food for ant larvae (Elmes et al., 2004). The camouflage hypothesis could not, however, explain why survival was extremely low in "non-host" colonies under stress. If we admit that the larvae of *M. rebeli* passively acquire the smell of a colony, then they should parasitize with the same success any species of the genus *Myrmica*, having passed the initial period of integration (Elmes et al., 2002). Therefore, it became clear that the larvae of *M. rebeli* are able to actively synthesize (mimicry *sensu* Dettner and Liepert, 1994) additional hydrocarbon compounds that increase and amplify the overlapping (mimicry) with the (host) model (Elmes et al., 2002, 2004; Schönrogge et al., 2004).

While the mechanisms allowing the chemical integration of cuckoo species of *Maculinea* butterflies (*M. rebeli* and *M. alcon*) have been extensively explored, only a few studies have investigated those evolved by predatory species. Data on the CHC profiles have shown that the post-adoption larvae of predatory *M. teleius* do not achieve the same level of chemical integration as cuckoo caterpillars. In a study conducted on two Polish populations the level of chemical similarity of the parasite caterpillars varied between 32 and 60% depending on the host species considered (Witek et al., 2013). This is in line with the fact that *M. teleius* is the most generalist species of the genus and is usually less locally specific to single *Myrmica* host (Witek et al., 2014; Tartally et al., 2019). The highest degree of chemical similarity was found within the host colonies of *M. rubra* at both investigated sites, suggesting that an ancestral association with this *Myrmica* species may exist. Differently from cuckoo *M. alcon*, whose larvae acquire a limited number of CHCs (at maximum 28 peaks in the post-adoption phase), *M. teleius* caterpillars showed very complex profiles which ranged from 57 CHC peaks to more than 70, i.e., almost double than shown in the CHC profile of *Myrmica* workers. These results, even if derived from a limited number of samples, suggest that the predatory species may have evolved a different mechanism of integration, possibly based on the acquisition of compounds by contact with the host species or by feeding on the ant brood (Witek et al., 2013).

On the contrary, the less studied *Maculinea* species, *M. nausithous*, is known to parasitize primarily a single host (*My rubra*) throughout its distribution range, from Western Europe to Southern Siberia and Mongolia (Witek et al., 2014; Tartally et al., 2019). Nevertheless, *M. nausithous* caterpillars do not acquire the same degree of chemical similarity as *M. alcon* does within its host colonies. This level (43%) is also lower than that of the *M. teleius* larvae, sharing the same *M. rubra* colonies, but higher than *M. teleius* caterpillars exploiting other *Myrmica* ants (Witek et al., 2013). In addition, its CHC profile seems intermediate between those of cuckoo and predatory species, suggesting that it may have evolved an intermediate strategy based both on mimicry of the host CHC profile and on chemical camouflage (Patricelli et al., 2010). Although still untested, the latter hypothesis finds some support in large-scale ecological studies, as *M. nausithous* shows intermediate host-specify patterns, differing form predatory species in coevolutionary trajectories and from cuckoos in local adaptation strategies (Tartally et al., 2019).

VIBROACOUSTIC SIGNALS

Insect communication can also be achieved by generating mechanical signals that cause a perturbation of the conveying medium. Compared to chemicals, vibroacoustic cues allow sending rapid, directional and quickly adjustable signals at both short or long-range (Frings and Frings, 1958; Hunt and Richard, 2013).

In ants, vibroacoustic communication is currently known to play important roles in colony life, such as in defense and signaling of alarm, territory, mating, rescue, recruitment, and social or caste status (Hölldobler and Wilson, 1990; Hickling and Brown, 2000; Casacci et al., 2013; Schönrogge et al., 2017) and may have evolved independently several times (Golden and Hill, 2016).

For ants, the simplest way for producing vibrations is the substrate tapping with part of the exoskeleton (i.e., “drumming”). However, specialized stridulatory organs (**Figure 2F**) occur in five subfamilies: Ponerinae, Pseudomyrmecinae, Myrmicinae, Ectomminae, and Nothomyrmecinae (Hunt and Richard, 2013; Golden and Hill, 2016; Schönrogge et al., 2017). The signal (stridulation) is produced by rubbing two body parts together, a scraper (*plectrum*) on a series of ridges (*pars stridens*) (Hunt and Richard, 2013; Golden and Hill, 2016).

A similar mechanism involving slightly different stridulatory organs (**Figure 2E**) produces calls in butterfly pupae (Alvarez et al., 2014; Dolle et al., 2018), while the modalities of larval sounds emissions are more diversified and not yet fully unraveled (**Figure 2D**) (except for some riodinids which possess specialized structures called vibratory papillae (DeVries, 1991b; Schönrogge et al., 2017).

In several butterfly species the emission of sounds can act as generic scaring or deterrent signals against enemies (Downey, 1966). In the case of lycaenids, in contrast, larval and pupal sounds may have taken up a role comparable to that of chemical signals in fostering interactions with ants (Downey, 1966; DeVries, 1990; Travassos and Pierce, 2000).

To assess the function of sounds in ant-butterfly symbioses it is sometimes possible to artificially prevent caterpillars from producing vibroacoustic signals and to check for changes in ant behavior. The first experimental evidence that vibroacoustic signals are used by butterfly larvae to attract ants was provided for the riodinid *Thisbe irenea*. Calling larvae were indeed tended by a higher number of workers than muted caterpillars (DeVries, 1991a). Ten years later, Travassos and Pierce (Travassos and Pierce, 2000) demonstrated that also larvae and pupae of the obligate mutualist *Jalmenus evagoras* use acoustic signals to enhance care from the *Iridomyrmex* ants to which they are associated. Larvae of *J. evagoras* are able to produce grunts, hisses, and drumming. While drumming occurs in both tended and untended larvae, hisses are emitted only during the first minutes after encountering a worker, and grunts are produced during all the ant attendance period (Travassos and Pierce, 2000). The positive correlation between the number of calls produced and the weight of the emitting pupa is considered to be an honest signal for ants to invest in a good quality and healthy resource. *J. evagoras* juveniles produce a complex repertoire of calls, which can be modulated to attract higher or lesser numbers of guarding workers and varied according to context, suggesting the evolution of a fine-tuned system of communication with their host ants.

As for butterfly parasitic interactions, clear-cut evidence of the role of vibroacoustic signals is only documented in *Maculinea* species where acoustical mimicry is employed along with other chemical and behavioral adaptations to deceive host ants and exploit nest resources (Barbero et al., 2009a,b, 2012; Thomas et al., 2010; Settele et al., 2011; Sala et al., 2014; Barbero and Casacci, 2015). The function of vibroacoustic communication in this model system was explored using another approach with respect to previous works (artificially muted individuals). After recordings, acoustic parameters were compared between parasite juveniles and their host ants to asses if any degree of mimicry occurs. Afterwards, sound stimuli were played back to ants to evaluate the behavioral responses of workers (Barbero et al., 2009b). In 2009, the first case of vibroacoustic mimicry in a social parasite of ants was discovered (Barbero et al., 2009b). Authors showed that larvae and pupae of *M. rebeli* are able to emit stridulations much more similar to those produced by *Myrmica schencki* queens, than to those of worker ants. Although occurring (see Chemical Signals), chemical mimicry does not explain how these butterfly parasites achieve a high social level in the colony hierarchy to such an extent that they are fed or rescued in preference to the ant brood (Thomas and Elmes, 1998). Instead, playback experiments revealed that the emission of queen-like calls allow the butterfly parasite to obtain a "royal" status, enhancing in workers the same degree of attendance and attention as queens do. In response to *M. rebeli* and queen vibroacoustic stimuli, ant workers tapped with antennae, gathered together around the speaker and showed guarding behaviors more frequently than when control or worker signals were played.

The ability to mimic queen sounds was assessed also in other predatory or cuckoo species i.e., *M. arion* populations hosted by *Myrmica sabuleti* (Barbero et al., 2009a) as well as in *M. alcon* and *M. teleius* hosted by *Myrmica scabrinodis* (Sala et al., 2014). Playback bioassays were performed to compare the function of vibroacoustic signals between the two lifestyles (cuckoo vs. predatory) in distinct stages of the biological cycle (Sala et al., 2014). For both the species, vibroacoustic signals change along with larval development and cause different amounts and variety of ant responses. In predatory species, the queen-like calls produced by early, just-dropped, larvae elicited the strongest ant response, thus contributing to enhance their retrieval by ants. Vibroacoustic stimuli probably serve to improve the supposed (but this requires formal testing) weak chemical mimicry shown by predatory species, which leads to very long adoption rituals (Fiedler, 1990; Sala et al., 2014). In contrast, cuckoo species primarily use vibroacoustic signals in the post-adoption phase to strengthen their social status and become fully integrated in the colony to outcompete ant brood for the same resources (Elmes et al., 1991; Sala et al., 2014).

Research on the vibroacoustic behavior in the *Maculinea-Myrmica* system has provided the first evidence that, at least in *Myrmica* ants, vibroacoustic cues are more than generic alarm signals and also convey information on the social rank of the emitting individual. The parasite mimics the intraspecific communication signals of its host, either to become intimately integrated within the colony, like in the cuckoo species, or just to enhance other channels of communication that might not be fully developed, like in the case of pre-adoption predatory larvae.

Although vibroacoustic signals can function as very precise interspecific messages (*Maculinea* and *Jalmenus* butterflies), the ability of producing calls is not limited to ant-associated lycaenid larvae (Alvarez et al., 2014; Schönrogge et al., 2017), since it is also present in species not associated with ants (myrmecoxenous) (Alvarez et al., 2014; Riva et al., 2017). Fiedler (1992) has suggested that the ability to produce vibroacoustic calls could be widespread in lycaenid species, but while myrmecoxenous species produce simple calls as a response to disturbance, myrmecophilous species emit more frequent and complex calls.

Analyzing several species of lycaenids, Riva et al. (2017) found that sounds emitted are strictly species-specific, but overall those produced by species associated with ants are made of longer and less distant pulses than calls of ant-independent species. Nevertheless, they did not point out differences in sound complexity. Multivariate comparisons of the vibroacoustic parameters of these calls were used to test the hypothesis that the similarity in lycaenid sounds is better predicted by the degree of ant association than by their phylogenetic distance (Schönrogge et al., 2017). In several instances, congeneric species made sounds that were much more similar to calls of distantly related species which showed the same type of ant association [myrmecophilous category as described by Fiedler (1991)]. Although the inclusion of further call analysis is required, authors suggested that the ability to produce vibroacoustic signals is a preadaptation to myrmecophily (Schönrogge et al., 2017).

In this framework, myrmecoxenous species could have maintained the ability to emit vibroacoustic stimuli either to repel natural enemies (Bura et al., 2009, 2011), or conspecifics sharing the same host plant at high densities, as observed in some butterflies and moths (Yack et al., 2001; Riva et al., 2017).

FUTURE DIRECTIONS

Eavesdropping

A complex communication system coordinates a large number of individuals in the collective decision-making process and maintains the social organization ensuring the ant ecological dominance (Wilson, 1985). Since by definition communication involves an emitter and a receiver, information in signals can be detected and exploited by a third "unplanned" individual other than the primary target (Peake, 2005). Yet, the *intraspecific* exchange of chemical or vibroacoustic signals of ants can be eavesdropped by commensal, mutualistic, or parasitic organisms, thereby, respectively, generating benefits and costs. Among myrmecophilous lycaenids, caterpillars of two parasitic species, *Euliphyra mirifica* and *E. leucyana*, intercept and follow trail pheromones released by arboreal weaver ant, *Oecophylla longinoda*, to locate their host ant nests (Dejean and Beugnon, 1996). Similarly, it is suggested that the first instar larvae of a close related species, *Liphyra brassolis*, detect the ant nests of their host, *O. smaragdina*, following pheromone trails of ants after hatching in the vicinity of the nests. *L. brassolis* larvae should also use the same trails to move between nests after depleting the brood of a nest or after host nest translocation (Common and Waterhouse, 1981).

As extensively reviewed above, ants may also be used as oviposition cues by adult females of myrmecophilous butterflies, but only indirect behavioral evidences are available. Further studies should investigate what signals, either chemical, vibroacoustic, or visual (Mota and Oliveira, 2016) are eavesdropped by females searching for the optimal LHP and the functioning of sensory structures possibly located on antennae and ovipositors which are used to perceive host plants colonized by ants.

Eavesdroppers can also intercept signals exchanged between ants and other organisms, but true instances of *interspecific* eavesdropping are rarely identified in multitrophic associations involving myrmecophilous butterflies.

Mathew et al. (2008) envisaged that the calls emitted by *Feniseca tarquinius,* which represents the first reported case of a calling Miletinae butterfly, might use vibroacoustic mimicry to avoid predation by ants. Authors argued that these larvae mimic the vibroacoustic signals produced by the ant-associated wooly aphids upon which they feed (Mathew et al., 2008). Beyond using these vibroacoustic signals to appease ants, would it be possible that caterpillars may use the putative vibroacoustic signals emitted by aphids to locate them? Unfortunately, no further studies were published, and data reported in this paper are not sufficient to support these speculations.

Although based on correlative data, field and laboratory experiments concur to suggest that orb-web spiders and braconid parasitoids, two natural enemies of the lycaenid *Jalmenus evagoras*, pinpoint their butterfly "preys" and increase their own success by exploiting the same ant volatiles that are supposedly used by the butterfly to detect the presence of their host ant, *Iridomyrmex mayri* (Elgar et al., 2016).

A well-documented case of *interspecific* eavesdropping is described in the second paragraph of this review. To locate the presence of the host ants and select the ideal oviposition site, *Maculinea arion* females follow signals emitted by their food plants responding and interacting with ants (Patricelli et al., 2015).

The paucity of robust evidence for *interspecific* eavesdropping is probably due to the lack of a community context approach in the study of multitrophic associations. Several studies focused on pairwise relations and do not consider other signals involved in levels not necessarily linked by trophic relations. For instance, it is expected that parasitoids of *Maculinea* butterflies exploit some form of *interspecific* eavesdropping to identify the presence of the host larvae inside the ant nest or within the flower buds. By releasing chemical compounds which elicit aggressive behavior and combats in workers, *Ichneumon* wasps create a safe route to brood chambers where they attack *Maculinea* cuckoo larvae (Thomas et al., 2002). They locate *Myrmica* colonies by ant-odor cues, but only enter those nests which host *Maculinea* larvae (Thomas and Elmes, 1993). How do they detect the butterfly larva inside the chamber in such a "chemical fuzziness?" Could it be possible that these parasitoids are able to perceive some components of *Maculinea* calls which is similar, but not entirely identical to the ant stridulations?

Maculinea predatory species, instead, are targeted by *Neotypus* parasitoids when they are still on their food plants (Tartally, 2005). Are these parasitoids able to perceive some plant response to egg-laying of *Maculinea* butterflies and use these signals to locate their own oviposition site?

Research considering the inclusive variation of costs and benefits in each combination of distinct trophic and information networks would be timely and would allow getting crucial insights on all the possible outcomes as well as on co-evolutionary dynamics in myrmecophilous systems.

Multimodal Communication

Another issue concerns the context, meaning, and function of multimodal signaling. It has been suggested that ants can use several signals (visual, tactile, and chemical) in peculiar intra-colony contexts to cause a prompt reaction in nestmates (Hölldobler and Wilson, 1990). Nonetheless, we possess little knowledge about the possibility that myrmecophilous organisms might manipulate ant behavior using multiple cues. Exploring the use of CHCs and vibrations in *Maculinea* species, authors collected increasing evidence that both types of signals are important and play distinct functions in different phases of the life cycle (**Figure 3**). Outside the colony, mimetic cuticular profiles, although simple, promote the adoption of *Maculinea* cuckoo larvae which usually occurs in few seconds after the first contact with the foraging ants; in predatory species, there are lines of evidence that this process could be fostered by vibroacoustic emissions (Sala et al., 2014). Since the adoption process can last for hours, predatory larvae may possess less mimetic cuticular profiles than cuckoo caterpillars and may have evolved more efficient vibroacoustic signals to compensate the chemical deficiency. Inside the nest, the CHC profile of parasites increase their resemblance with the host profile (Witek et al., 2013), but this achievement may not be sufficient to sneak undisturbed into the larval chamber to feed on the colony brood. The vibroacoustic signals, whose resemblance to the host stridulations arises inside the colony, could act together with chemical signals increasing the ant response and therefore the parasite acceptance.

It is likely that the chemical and vibroacoustic signals have evolved independently and in the myrmecophilous parasite species most intimately linked to their host they have begun to operate in a multimodal way to allow the parasite to reach the highest level of integration within the colony. In other myrmecophilous organisms, commensal or mutualistic species, it is possible that the interaction with the host species is primarily reinforced by chemical signals and rewarding substances, while only slightly modulated by vibroacoustic cues as calling signals.

There are still many aspects to be completely discerned and it is clear that future research should try to better categorize signals collecting data on ant responses both to the multimodal composite signal and to each unimodal element. Testing the response to both chemical and vibroacoustic emissions separately would be necessary to determine whether the components produce the same or different outcomes (Partan and Marler, 2005). While the response of a colony to "isolated" vibroacoustic stimuli has been assessed in playback experiments, bioassays testing the role of CHC employing dummies are almost lacking. In addition, investigating ant behavioral responses to CHC-painted dummies supplemented with vibroacoustic signals could allow to gather robust insights on how distinct channels of communication, such as the chemical and the vibroacoustic one, may interact.

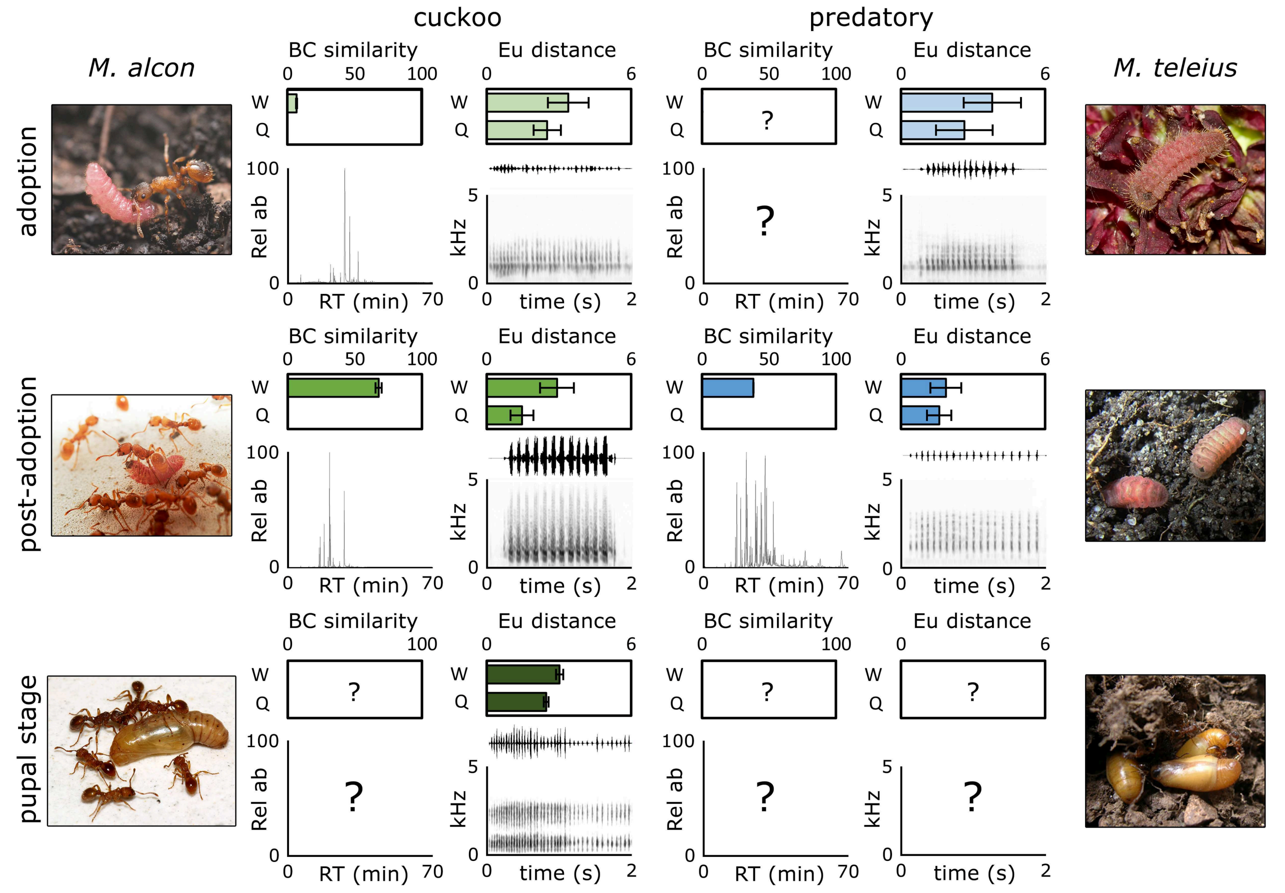

FIGURE 3 | Comparison of chemicals and vibroacoustic patterns used by cuckoo and predatory caterpillars of *Maculinea* butterflies during pre- and post-adoption and the pupal phase to deceive their *Myrmica* host ants. Bray-Curtis (BC) similarities calculated on relative abundances (Rel ab) of cuticular hydrocarbons of parasitic caterpillars and host workers in pre-adoption (Schönrogge et al., 2004) and during the integration phase within host colonies (Witek et al., 2013) are reported as well as Euclidean (Eu) distances between the vibroacoustic patterns of the host queens (Q) and workers (W) and the parasite in pre- and post-adoption (Sala et al., 2014) and during the pupal stage (Barbero et al., 2009b). Pre-adoption chemical data and vibroacoustic patterns for pupal stage refer to *M. rebeli* and its host ant *M. schencki*. Chemical and vibroacoustic patterns showed for the post-adoption phase refer to *M. alcon* and *M. teleius* exploiting *M. scabrinodis*.

CONCLUDING REMARKS

The ant-association patterns are considered "a template" for the evolutionary radiation of lycaenid butterflies (Pierce et al., 2002). On the other hand, communication, which can be considered as an extended phenotype (*sensu* Dawkins, 1982) of the colony, may have played a pivotal role in the evolution of social insects. In our review, we tried to point out that the amazing complexity of signaling between myrmecophiles and their attendant ants could have boosted the evolutionary onsets of specialized life cycles thereby acting as a source of increasing diversity within butterflies.

AUTHOR CONTRIBUTIONS

FB led the writing of the manuscript to which all authors contributed and gave final approval for publication.

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REFERENCES

Agrawal, A. A., and Fordyce, J. A. (2000). Induced indirect defence in a lycaenid-ant association, the regulation of a resource in a mutualism. *Proc. R. Soc. Lond. B* 267, 1857–1861. doi: 10.1098/rspb.2000.1221

Akino, T., Knapp, J. J., Thomas, J. A., and Elmes, G. W. (1999). Chemical mimicry and host specificity in the butterfly *Maculinea rebeli*, a social parasite of *Myrmica* ant colonies. *Proc. R. Soc. Lond. B* 266, 1419–1426. doi: 10.1098/rspb.1999.0796

Als, T. D., Nash, D. R., and Boomsma, J. J. (2001). Adoption of parasitic *Maculinea alcon* caterpillars (Lepidoptera: Lycaenidae). by three *Myrmica* ant species. *Anim. Behav*. 62, 99–106. doi: 10.1006/anbe.2001.1716

Alvarez, M., Munguira, M. L., and Martínez-Ibáñez, M. D. (2014). Comparative study of the morphology of stridulatory organs of the Iberian lycaenid butterfly pupae (Lepidoptera). *J. Morph*. 275, 414–430. doi: 10.1002/jmor.20224

Atsatt, P. R. (1981). Lycaenid butterflies and ants: selection for enemy-free space. *Am. Nat*. 118, 638–654. doi: 10.1086/283859

Axén, A. H., Leimar, O., and Hoffman, V. (1996). Signalling in a mutualistic interaction. *Anim. Behav*. 52, 321–333. doi: 10.1006/anbe.1996.0178

Axén, A. H., and Pierce, N. E. (1998). Aggregation as a cost-reducing strategy for lycaenid larvae. *Behav. Ecol*. 9, 109–115. doi: 10.1093/beheco/9.2.109

Bächtold, A., Alves-Silva, E., Kaminski, L. A., and Del-Claro, K. (2014). The role of tending ants in host plant selection and egg parasitism of two facultative myrmecophilous butterflies. *Naturwissenschaften* 101, 913–919. doi: 10.1007/s00114-014-1232-9

Barbero, F. (2016). Cuticular lipids as a cross-talk among ants, plants and butterflies. *Int. J. Mol. Sci*. 17:1966. doi: 10.3390/ijms17121966

Barbero, F., Bonelli, S., Thomas, J. A., Balletto, E., and Schönrogge, K. (2009a). Acoustical mimicry in a predatory social parasite of ants. *J. Exp. Biol*. 212, 4084–4090. doi: 10.1242/jeb.032912

Barbero, F., and Casacci, L. P. (2015). Butterflies that trick ants with sound. *Phys. Today* 68, 64–65. doi: 10.1063/PT.3.2757

Barbero, F., Patricelli, D., Witek, M., Balletto, E., Casacci, L. P., Sala, M., et al. (2012). *Myrmica* ants and their butterfly parasites with special focus on the acoustic communication. *Psyche* 2012:725237. doi: 10.1155/2012/725237

Barbero, F., Thomas, J. A., Bonelli, S., Balletto, E., and Schönrogge, K. (2009b). Queen ants make distinctive sounds that are mimicked by a butterfly social parasite. *Science* 323, 782–785. doi: 10.1126/science.1163583

Blüthgen, N. E., Stork, N., and Fiedler, K. (2004). Bottom-up control and co-occurrence in complex communities: honeydew and nectar determine a rainforest ant mosaic. *Oikos* 106, 344–358. doi: 10.1111/j.0030-1299.2004.12687.x

Bos, N., Lefevre, T., Jensen, A., and D'ettorre, P. (2012). Sick ants become unsociable. *J. Evol. Biol*. 25, 342–351. doi: 10.1111/j.1420-9101.2011.02425.x

Bura, V. L., Fleming, A. J., and Yack, J. E. (2009). What's the buzz? Ultrasonic and sonic warning signals in caterpillars of the great peacock moth (*Saturnia pyri*). *Naturwissenschaften* 96, 713–718. doi: 10.1007/s00114-009-0527-8

Bura, V. L., Rohwer, V. G., Martin, P. R., and Yack, J. E. (2011). Whistling in caterpillars (*Amorpha juglandis*, Bombycoidea): sound-producing mechanism and function. *J. Exp. Biol*. 214, 30–37. doi: 10.1242/jeb.046805

Carleial, S., Maurel, N., van Kleunen, M., and Stift, M. (2018). Oviposition by the mountain alcon blue butterfly increases with host plant flower number and host ant abundance. *Basic Appl. Ecol*. 28, 87–96. doi: 10.1016/j.baae.2018.02.008

Casacci, L. P., Schönrogge, K., Thomas, J. A., Balletto, E., Bonelli, S., and Barbero, F. (2019). Host specificity pattern and chemical deception in a social parasite of ants. *Sci. Rep*. 9:1619. doi: 10.1038/s41598-018-38172-4

Casacci, L. P., Thomas, J. A., Sala, M., Treanor, D., Bonelli, S.,Balletto, E., et al. (2013). Ant pupae employ acoustics to communicate social status in their colony's hierarchy. *Curr.Biol*. 23, 323–327. doi: 10.1016/j.cub.2013.01.010

Chew, F. S., and Robbins, R. K. (1984). "Egg-laying in butterflies," in *The Biology of Butterflies*, eds R. I. Vane-Wright, and P. R. Ackery (London: Academic), 65–79.

Claassens, A., and Dickson, C. (1977). A study of the myrmecophilous behaviour of the immature stages of *Aloeides thryra* (L.) (Lep.: Lycaenidae) with special reference to the function of the retractile tubercles and with additional notes of the general biology of the species. *Entomol. Rec. J. Var*. 89, 253–258.

Common, I. F. B., and Waterhouse, D. F. (1981). *Butterflies of Australia. Revised Edition* (Sydney: Angus & Robertson).

Cottrell, C. B. (1984). Aphytophagy in butterflies - its relationship to myrmecophily. *Zool. J. Linn. Soc*. 80, 1–57. doi: 10.1111/j.1096-3642.1984.tb02318.x

Cushman, J. H., Rashbrook, V. K., and Beattie, A. J. (1994). Assessing benefits to both participants in a lycaenid-ant association. *Ecology* 75, 1031–1041. doi: 10.2307/1939427

Czekes, Z., Marko, B., Nash, D. R., Ferencz, M., Lazar, B., and Rakosy, L. (2014). Differences in oviposition strategies between two ecotypes of the endangered myrmecophilous butterfly *Maculinea alcon* (Lepidoptera: Lycaenidae) under unique syntopic conditions. *Insect Conserv. Divers*. 7, 122–131. doi: 10.1111/icad.12041

Dani, F. R., Jones, G. R., Destri, S., Spencer, S. H., and Turillazzi, S. (2001). Deciphering the recognition signature within the cuticular chemical profile of paper wasps. *Anim. Behav*. 62, 165–171. doi: 10.1006/anbe.2001.1714

Daniels, H., Gottsberger, G., and Fiedler, K. (2005). Nutrient composition of larval nectar secretions from three species of myrmecophilous butterflies. *J. Chem. Ecol*. 31, 2805–2821. doi: 10.1007/s10886-005-8395-y

Davidson, D. W., Cook, S. C., Snelling, R. R., and Chua, T. H. (2003). Explaining the abundance of ants in lowland tropical rainforest canopies. *Science* 300, 969–972. doi: 10.1126/science.1082074

Dawkins, R. (1982). *The Extended Phenotype*. Vol. 8. Oxford: Oxford University Press, 287.

Dejean, A., and Beugnon, G. (1996). Host-ant trail following by myrmecophilous larvae of Liphyrinae (Lepidoptera, Lycaenidae). *Oecologia* 106, 57–62. doi: 10.1007/BF00334407

Dettner, K., and Liepert, C. (1994). Chemical mimicry and camouflage. *Annu. Rev. Entomol*. 39, 129–154. doi: 10.1146/annurev.en.39.010194.001021

DeVries, P.J. (1988). The larval ant-organs of Thisbe irenea (Lepidoptera: *Riodinidae*). and their effects upon attending ants. *Zool. J. Linn. Soc*. 94, 379–393. doi: 10.1111/j.1096-3642.1988.tb01201.x

DeVries, P. J. (1984). Of crazy-ants and curetinae: are curetis butterflies tended by ants? *Zool. J. Linn. Soc*. 80, 59–66. doi: 10.1111/j.1096-3642.1984.tb02319.x

DeVries, P. J. (1990). Enhancement of symbioses between butterfly caterpillars and ants by vibrational communication. *Science* 248, 1104–1106. doi: 10.1126/science.248.4959.1104

DeVries, P. J. (1991a). Mutualism between thisbe-irenea butterflies and ants, and the role of ant ecology in the evolution of larval-ant associations. *Biol. J. Linn. Soc. Lond*. 43, 179–195. doi: 10.1111/j.1095-8312.1991.tb00592.x

DeVries, P. J. (1991b). Call production by myrmecophilous riodinid and lycaenid butterfly caterpillars (Lepidoptera).: morphological, acoustical, functional, and evolutionary patterns. *Am. Mus. Novit*. 3025, 1–23.

DeVries, P. J. (1991c). "Evolutionary and ecological patterns in myrmecophilous-riodinid butterflies," in *Ant - Plant Interactions*, eds C.R. Huxley and D.F. Cutler (Oxford: Oxford University Press), 143–156.

Dicke, M. (2000). Chemical ecology of host-plant selection by herbivorous arthropods: a multitrophic perspective. *Biochem. System. Ecol*. 28, 601–617. doi: 10.1016/S0305-1978(99)00106-4

Dolle, P., Klein, P., Fischer, O. W., Schnitzler, H.-U., Gilbert, L. E., and Boppré, M. (2018). Twittering pupae of papilionid and nymphalid butterflies (Lepidoptera): novel structures and sounds. *Ann. Entomol. Soc. Amer*. 111, 341–354. doi: 10.1093/aesa/say029

Donisthorpe, H. S. J. K. (1927). *The Guests of British Ants*. London: Routledge.

Downey, J. C. (1966). Sound production in pupae of Lycaenidae. *J. Lepid. Soc*. 20, 129–155.

Elgar, M. A., Nash, D. R., and Pierce, N. E. (2016). Eavesdropping on cooperative communication within an ant-butterfly mutualism. *Sci. Nat*. 103:84. doi: 10.1007/s00114-016-1409-5

Elgar, M. A., and Pierce, N. E. (1988). "Mating success and fecundity in an ant-tended lycaenid butterfly" in *Reproductive Success: Studies of Selection and Adaptation in Contrasting Breeding Systems* ed T. H. Clutton-Brock (Chicago, IL: Chicago University Press), 59–75.

Elmes, G., Akino, T., Thomas, J., Clarke, R., and Knapp, J. (2002). Interspecific differences in cuticular hydrocarbon profiles of *Myrmica* ants are sufficiently consistent to explain host specificity by *Maculinea* (large blue) butterflies. *Oecologia* 130, 525–535. doi: 10.1007/s00442-001-0857-5

Elmes, G., Wardlaw, J., and Thomas, J. (1991). Larvae of *Maculinea rebeli*, a large-blue butterfly and their *Myrmica* host ants: patterns of caterpillar growth and survival. *J. Zool*. 224, 79–92. doi: 10.1111/j.1469-7998.1991.tb04789.x

Elmes, G. W., Wardlaw, J. C., Schönrogge, K., Thomas, J. A., and Clarke, R. T. (2004). Food stress causes differential survival of socially parasitic caterpillars of *Maculinea rebeli* integrated in colonies of host and non-host *Myrmica* ant species. *Entomol. Exp. Appl*. 110, 53–63. doi: 10.1111/j.0013-8703.2004.00121.x

Fiedler, K. (1990). New information on the biology of *Maculinea nausithous* and *M. teleius* (Lepidoptera: Lycaenidae). *Nota Lepid*. 12, 246–256.

Fiedler, K. (1991). Systematic, evolutionary, and ecological implications of myrmecophily within the Lycaenidae (Insecta: Lepidoptera: Papilionoidea). *Bonn. Zool. Monogr*. 31, 1–210.

Fiedler, K. (1992). Recent contributions to the behavioural ecology and evolution of lycaenid-ant associations (Lepidoptera, Lycaenidae). *Nota Lepid.* 4, 11–13.

Fiedler, K. (1994). Lycaenid butterflies and plants: is myrmecophily associated with amplified hostplant diversity? *Ecol. Entomol*. 19, 79–82. doi: 10.1111/j.1365-2311.1994.tb00393.x

Fiedler, K. (1998). Geographical patterns in life-history traits of Lycaenidae butterflies: ecological and evolutionary implications. *Zoology* 100, 336–347.

Fiedler, K., and Maschwitz, U. (1988). Functional analysis of the myrmecophilous relationships between ants (Hymenoptera: Formicidae). and lycaenids (Lepidoptera: Lycaenidae). *Oecologia* 75, 204–206. doi: 10.1007/BF00378598

Fiedler, K., and Maschwitz, U. (1989a). Adult myrmecophily in butterflies: the role of the ant *Anoplolepis longipes* in the feeding and oviposition behaviour of Allotinus unicolor (Lepidoptera, Lycaenidae) *Lepid. Sci.* 40, 241–251.

Fiedler, K., and Maschwitz, U. (1989b). The symbiosis between the weaver ant, *Oecophylla smaragdina*, and *Anthene emolus*, an obligate myrmecophilous lycaenid butterfly. *J. Nat. Hist*. 23, 833–846. doi: 10.1080/00222938900770441

Fiedler, K., and Maschwitz, U. (1989c). Functional analysis of the myrmecophilous relationships between ants (Hymenoptera: Formicidae). and Lycaenids (Lepidoptera: Lycaenidae). I. Release of food recruitment in ants by lycaenid larvae and pupae. *Ethology* 80, 71–80. doi: 10.1111/j.1439-0310.1989.tb00730.x

Fraser, A., Tregenza, T., Wedell, N., Elgar, M., and Pierce, N. (2002). Oviposition tests of ant preference in a myrmecophilous butterfly. *J. Evol. Biol*. 15, 861–870. doi: 10.1046/j.1420-9101.2002.00434.x

Frings, H., and Frings, M. (1958). Uses of sounds by insects. *Annu. Rev. Entomol*. 3, 87–106. doi: 10.1146/annurev.en.03.010158.000511

Fürst, M. A., and Nash, D. R. (2009). Host ant independent oviposition in the parasitic butterfly *Maculinea alcon*. *Biol. Lett*. 6, 174–176. doi: 10.1098/rsbl.2009.0730

Gibbs, A. G. (1998). Water-proofing properties of cuticular lipids. *Amer. Zool*. 38, 471–482. doi: 10.1093/icb/38.3.471

Gnatzy, W., Jatho, M., Kleinteich, T., Gorb, S., and Hustert, R. (2017). The eversible tentacle organs of *Polyommatus* caterpillars (Lepidoptera, Lycaenidae): morphology, fine structure, sensory supply and functional aspects. *Arthropod. Struct. Develop*. 46, 788–804. doi: 10.1016/j.asd.2017.10.003

Golden, T., and Hill, P. (2016). The evolution of stridulatory communication in ants, revisited. *Ins. Soc*. 63, 309–319. doi: 10.1007/s00040-016-0470-6

Guerrieri, F. J., Nehring, V., Jørgensen, C. G., Nielsen, J., Galizia, C. G., and d'Ettorre, P. (2009). Ants recognize foes and not friends. *Proc. R. Soc. Lond. B* 276, 2461–2468. doi: 10.1098/rspb.2008.1860

Harvey, D. (1987). *The higher classification of the Riodinidae* (PhD dissertation). University of Texas, Austin, TX, United States.

Henning, S. F. (1983). Chemical communication between Iycaenid larvae (Lepidoptera: Lycaenidae) and ants (Hymenoptera: Formicidae). *J Entomol. Soc. South. Afr*. 46, 341–366.

Hickling, R., and Brown, R. L. (2000). Analysis of acoustic communication by ants. *J Acoust. Soc. Am*. 108, 1920–1929. doi: 10.1121/1.1290515

Hinton, H. E. (1951). Myrmecophilous Lycaenidae and other Lepidoptera - a summary. *Proc. Trans. South Lond. Entomol. Nat. Hist. Soc.* 1949–50, 111–175.

Hojo, M. K., Pierce, N. E., and Tsuji, K. (2015). Lycaenid caterpillar secretions manipulate attendant ant behavior. *Curr. Biol*. 25, 2260–2264. doi: 10.1016/j.cub.2015.07.016

Hojo, M. K., Wada-Katsumata, A., Akino, T., Yamaguchi, S., Ozaki, M., and Yamaoka, R. (2009). Chemical disguise as particular caste of host ants in the ant inquiline parasite *Niphanda fusca* (Lepidoptera: Lycaenidae). *Proc. R. Soc. Lond. B* 276, 551–558. doi: 10.1098/rspb.2008.1064

Hojo, M. K., Yamamoto, A., Akino, T., Tsuji, K., and Yamaoka, R. (2014). Ants use partner specific odors to learn to recognize a mutualistic partner. *PLoS ONE* 9:e86054. doi: 10.1371/journal.pone.0086054

Hölldobler, B., and Wilson, E. O. (1990). *The Ants*. Berlin; Heidelberg: Springer Verlag. doi: 10.1007/978-3-662-10306-7

Hunt, J., and Richard, F.-J. (2013). Intracolony vibroacoustic communication in social insects. *Ins. Soc*. 60, 403–417. doi: 10.1007/s00040-013-0311-9

Jordano, D., Rodriguez, J., Thomas, C., and Haeger, J. F. (1992). The distribution and density of a lycaenid butterfly in relation to *Lasius* ants. *Oecologia* 91, 439–446. doi: 10.1007/BF00317635

Kaminski, L. A., Mota, L. L., Freitas, A. V. L., and Moreira, G. R. P. (2013). Two ways to be a myrmecophilous butterfly: natural history and comparative immature-stage morphology of two species of Theope (Lepidoptera: *Riodinidae*). *Biol. J. Linn. Soc. Lond.* 108, 844–870. doi: 10.1111/bij.12014

Kitching, R. (1987). Aspects of the natural history of the lycaenid butterfly Allotinus major in Sulawesi. *J. Nat. Hist*. 21, 535–544. doi: 10.1080/00222938700770311

Kitching, R., and Luke, B. (1985). The myrmecophilous organs of the larvae of some British Lycaenidae (Lepidoptera): a comparative study. *J. Nat. Hist*. 19, 259–276. doi: 10.1080/00222938500770211

Leimar, O., and Axén, A. H. (1993). Strategic behaviour in an interspecific mutualism: interactions between lycaenid larvae and ants. *Anim. Behav*. 46, 1177–1182. doi: 10.1006/anbe.1993.1307

Lenoir, A., D'Ettorre, P., Errard, C., and Hefetz, A. (2001). Chemical ecology and social parasitism in ants. *Annu. Rev. Entomol*. 46, 573–599. doi: 10.1146/annurev.ento.46.1.573

Malicky, H. (1969). Versuch einer Analyse der ökologischen Beziehungen zwischen Lycaeniden (Lepidoptera). und Formiciden (Hymenoptera). *Tijdschrift voor Entomologie* 112, 213–298.

Mannino, G., Abdi, G., Maffei, M. E., and Barbero, F. (2018). Origanum vulgare terpenoids modulate *Myrmica scabrinodis* brain biogenic amines and ant behaviour. *PLoS ONE* 13:e0209047. doi: 10.1371/journal.pone.0209047

Maschwitz, U., Schroth, M., Hanel, H., and Tho, Y. (1985). Aspects of the larval biology of myrmecophilous lycaenids from West Malaysia (Lepidoptera*). Nachr. ent. Ver. Apollo. Frankfurt*. 6, 181–200.

Maschwitz, U., Wüst, M., and Schurian, K. (1975). Bläulingsraupen als Zuckerlieferanten für Ameisen. *Oecologia* 18, 17–21. doi: 10.1007/BF00350631

Mathew, J., Travassos, M., Canfield, M., Murawski, D., Kitching, R., and Pierce, N. (2008). The singing reaper: diet, morphology and vibrational signaling in the nearctic species *Feniseca tarquinius* (Lepidoptera: Lycaenidae, Miletinae). *Trop. Lepid. Res*. 18, 24–29.

Mizuno, T., Hagiwara, Y., and Akino, T. (2018). Chemical tactic of facultative myrmecophilous lycaenid pupa to suppress ant aggression. *Chemoecology* 28, 173–182. doi: 10.1007/s00049-018-0270-8

Moreau, C. S., Bell, C. D., Vila, R., Archibald, S. B., and Pierce, N. E. (2006). Phylogeny of the ants: diversification in the age of angiosperms. *Science* 312, 101–104. doi: 10.1126/science.1124891

Mota, L. L., and Oliveira, P. S. (2016). Myrmecophilous butterflies utilise ant–treehopper associations as visual cues for oviposition. *Ecol. Entomol*. 41, 338–343. doi: 10.1111/een.12302

Murray, D. P. (1935). *South African Butterflies: A Monograph of the Family Lycaenidae*. London: Staples Press.

Musche, M., Anton, C., Worgan, A., and Settele, J. (2006). No experimental evidence for host ant related oviposition in a parasitic butterfly. *J. Ins. Behav*. 19, 631–643. doi: 10.1007/s10905-006-9053-0

Nash, D. R., Als, T. D., Maile, R., Jones, G. R., and Boomsma, J. J. (2008). A mosaic of chemical coevolution in a large blue butterfly. *Science* 319, 88–90. doi: 10.1126/science.1149180

Nelson, D., and Blomquist, G. (1995). "Insect waxes," in *Waxes: Chemistry, Molecular Biology and Functions*, ed. R. J. Hamilton (Dundee: The Oily Press), 1–90.

New, T. R. (2017). *Mutualisms and Insect Conservation*. Berlin: Springer. doi: 10.1007/978-3-319-58292-4

Newcomer, E. J. (1912). Some observations on the relations of ants and lycaenid caterpillars, and a description of the relational organs of the latter. *J. N. Y. Entomol. Soc*. 20, 31–36.

Nomura, K. (1992). Problems in the symbiosis between the lycaenid butterfly larva, *Niphanda fusca* and *Camponotus japonicus* (1). *J. Lepid. Soc. Jpn*. 43, 138–143.

Omura, H., Watanabe, M., and Honda, K. (2009). Cuticular hydrocarbons of larva and pupa of Reverdin's blue, Lycaeides argyrognomon (Lycaenidae). and its tending ants. *Lepid. Sci.* 60, 203–210.

Partan, S. R., and Marler, P. (2005). Issues in the classification of multimodal communication signals. *Am. Nat*. 166, 231–245. doi: 10.1086/431246

Patricelli, D., Barbero, F., La Morgia, V., Casacci, L. P., Witek, M., Balletto, E., et al. (2011). To lay or not to lay: oviposition of *Maculinea arion* in relation to *Myrmica* ant presence and host plant phenology. *Anim. Behav*. 82, 791–799. doi: 10.1016/j.anbehav.2011.07.011

Patricelli, D., Barbero, F., Occhipinti, A., Bertea, C. M., Bonelli, S., Casacci, L. P., et al. (2015). Plant defences against ants provide a pathway to social parasitism in butterflies. *Proc. R. Soc. Lond. B* 282:20151111. doi: 10.1098/rspb.2015.1111

Patricelli, D., Witek, M., Barbero, F., Casacci, L. P., Bonelli, S., and Balletto, E. (2010). Evidence of high larval host ant (Hymenoptera: Formicidae). specificity in the first post-adoption phase for the myrmecophilous butterfly *Phengaris (Maculinea)*. nausithous (Lepidoptera: Lycaenidae). *Sociobiology* 55, 861–870.

Peake, T. M. (2005). "Eavesdropping in communication networks," in *Animal Communication Networks*, ed. P. K. McGregor (Cambridge: Cambridge University Press), 13–37. doi: 10.1017/CBO9780511610363.004

Pech, P., Fric, Z., Konvička, M., and Zrzavý, J. (2004). Phylogeny of *Maculinea* blues (Lepidoptera: Lycaenidae). based on morphological and ecological characters: evolution of parasitic myrmecophily. *Cladistics* 20, 362–375. doi: 10.1111/j.1096-0031.2004.00031.x

Pellissier, L., Litsios, G., Guisan, A., and Alvarez, N. (2012). Molecular substitution rate increases in myrmecophilous lycaenid butterflies (Lepidoptera). *Zool. Scripta*. 41, 651–658. doi: 10.1111/j.1463-6409.2012.00556.x

Pierce, N., Kitching, R., Buckley, R., Taylor, M., and Benbow, K. (1987). The costs and benefits of cooperation between the Australian lycaenid butterfly, *Jalmenus evagoras*, and its attendant ants. *Behav. Ecol. Sociobiol*. 21, 237–248. doi: 10.1007/BF00292505

Pierce, N. E. (1984). "Amplified species diversity: a case study of an Australian lycaenid butterfly and its attendant ants," in *The Biology of Butterflies*, eds R. I. Vane-Wright and P. R. Ackery (London: Academic), 197–200.

Pierce, N. E., Braby, M. F., Heath, A., Lohman, D. J., Mathew, J., Rand, D. B., et al. (2002). The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). *Annu. Rev. Entomol*. 47, 733–771. doi: 10.1146/annurev.ento.47.091201.145257

Pierce, N. E., and Elgar, M. A. (1985). The influence of ants on host plant selection by Jalmenus evagoras, a myrmecophilous lycaenid butterfly. *Behav. Ecol. Sociobiol*. 16, 209–222. doi: 10.1007/BF00310983

Pierce, N. E., and Nash, D. R. (1999). The imperial blue, *Jalmenus evagoras* (Lycaenidae). *Monogr. Austr. Lepid*. 6, 279–315.

Quilico, A., Piozzi, F., and Pavan, M. (1957). The structure of dendrolasin. *Tetrahedron* 1, 177–185. doi: 10.1016/0040-4020(57)88037-5

Renwick, J., and Chew, F. (1994). Oviposition behavior in Lepidoptera. *Annu. Rev. Entomol*. 39, 377–400. doi: 10.1146/annurev.en.39.010194.002113

Rico-Gray, V., and Oliveira, P. S. (2007). *The Ecology and Evolution of Ant-Plant Interactions*. Chicago, IL: University of Chicago Press. doi: 10.7208/chicago/9780226713540.001.0001

Riva, F., Barbero, F., Bonelli, S., Balletto, E., and Casacci, L. P. (2017). The acoustic repertoire of lycaenid butterfly larvae. *Bioacoustics* 26, 77–90. doi: 10.1080/09524622.2016.1197151

Rodrigues, D., Kaminski, L. A., Freitas, A. V., and Oliveira, P. S. (2010). Trade-offs underlying polyphagy in a facultative ant-tended florivorous butterfly: the role of host plant quality and enemy-free space. *Oecologia* 163, 719–728. doi: 10.1007/s00442-010-1626-0

Sala, M., Casacci, L. P., Balletto, E., Bonelli, S., and Barbero, F. (2014). Variation in butterfly larval acoustics as a strategy to infiltrate and exploit host ant colony resources. *PLoS ONE* 9:e94341. doi: 10.1371/journal.pone.0094341

Samson, P. (1989). Morphology and biology of *Acrodipsas illidgei* (waterhouse and lyell), a myrmecophagous lycaenid (lepidoptera: lycaenidae: theclinae). *Aust. J. Entomol*. 28, 161–168. doi: 10.1111/j.1440-6055.1989.tb00874.x

Sanetra, M., and Fiedler, K. (1996). Behaviour and morphology of an aphytophagous lycaenid caterpillar: Cigaritis (Apharitis) acamas Klug, 1834 (Lepidoptera: Lycaenidae). *Nota Lepid*. 18, 57–76.

Schär, S., Eastwood, R., Arnaldi, K. G., Talavera, G., Kaliszewska, Z. A., Boyle, J. H., et al. (2018). Ecological specialization is associated with genetic structure in the ant-associated butterfly family Lycaenidae. *Proc. R. Soc. Lond. B* 285:20181158. doi: 10.1098/rspb.2018.1158

Schönrogge, K., Barbero, F., Casacci, L. P., Settele, J., and Thomas, J. A. (2017). Acoustic communication within ant societies and its mimicry by mutualistic and socially parasitic myrmecophiles. *Anim. Behav*. 134, 249–256. doi: 10.1016/j.anbehav.2016.10.031

Schönrogge, K., Wardlaw, J., Peters, A., Everett, S., Thomas, J., and Elmes, G. (2004). Changes in chemical signature and host specificity from larval retrieval to full social integration in the myrmecophilous butterfly *Maculinea rebeli*. *J Chem. Ecol*. 30, 91–107. doi: 10.1023/B:JOEC.0000013184.18176.a9

Sendoya, S. F., Freitas, A. V., and Oliveira, P. S. (2009). Egg-laying butterflies distinguish predaceous ants by sight. *Am. Nat.* 174, 134–140. doi: 10.1086/599302

Settele, J., Barbero, F., Musche, M., Thomas, J. A., and Schönrogge, K. (2011). Singing the blues: from experimental biology to conservation application. *J. Exp. Biol*. 214, 1407–1410. doi: 10.1242/jeb.035329

Shields, O. (1989). World numbers of butterflies. *J. Lepid. Soc*. 43, 178–183.

Singer, T. L. (1998). Roles of hydrocarbons in the recognition systems of insects. *Am. Zool*. 38, 394–405. doi: 10.1093/icb/38.2.394

Solazzo, G., Seidelmann, K., Moritz, R. F. A., and Settele, J. (2014). Tetracosane on the cuticle of the parasitic butterfly Phengaris (*Maculinea*) nausithous triggers the first contact in the adoption process by *Myrmica rubra* foragers. *Physiol. Entomol*. 60, 57–64. doi: 10.1007/s00040-012-0265-3

Stadler, B., Kindlmann, P., Šmilauer, P., and Fiedler, K. (2003). A comparative analysis of morphological and ecological characters of European aphids and lycaenids in relation to ant attendance. *Oecologia* 135, 422–430. doi: 10.1007/s00442-003-1193-8

Tartally, A. (2005). Neotypus melanocephalus (Hymenoptera: Ichneumonidae): the first record of a parasitoid wasp attacking *Maculinea teleius* (Lycaenidae). *Nota Lepid*. 28, 65–57.

Tartally, A., Thomas, J. A., Anton, C., Balletto, E., Barbero, F., Bonelli, S., et al. (2019). Patterns of host use by brood parasitic *Maculinea* butterflies across Europe. *Philos. Trans. R. Soc. B* 374:20180202. doi: 10.1098/rstb.2018.0202

Thomas, J. (1984). The behaviour and habitat requirements of *Maculinea* nausithous (the dusky large blue butterfly) and *M.* teleius (the scarce large blue) in France. *Biol. Conserv*. 28, 325–347. doi: 10.1016/0006-3207(84)90040-5

Thomas, J., and Elmes, G. (1993). Specialized searching and the hostile use of allomones by a parasitoid whose host, the butterfly *Maculinea rebeli*, inhabits ant nests. *Anim. Behav*. 45, 593–602. doi: 10.1006/anbe.1993.1069

Thomas, J., Elmes, G., and Wardlaw, J. (1998). Polymorphic growth in larvae of the butterfly *Maculinea rebeli*, a social parasite of *Myrmica* ant colonies. *Proc. R. Soc. Lond. B* 265, 1895–1901. doi: 10.1098/rspb.1998.0517

Thomas, J., Knapp, J., Akino, T., Gerty, S., Wakamura, S., Simcox, D., et al. (2002). Insect communication: parasitoid secretions provoke ant warfare. *Nature* 417:505. doi: 10.1038/417505a

Thomas, J. A., and Elmes, G. W. (1998). Higher productivity at the cost of increased host-specificity when *Maculinea* butterfly larvae exploit ant colonies through trophallaxis rather than by predation. *Ecol. Entomol*. 23, 457–464. doi: 10.1046/j.1365-2311.1998.00153.x

Thomas, J. A., and Elmes, G. W. (2001). Food-plant niche selection rather than the presence of ant nests explains oviposition patterns in the myrmecophilous butterfly genus *Maculinea*. *Proc. R. Soc. Lond. B* 268, 471–477. doi: 10.1098/rspb.2000.1398

Thomas, J. A., Elmes, G. W., Sielezniew, M., Stankiewicz-Fiedurek, A., Simcox, D. J., Settele, J, et al. (2013). Mimetic host shifts in an endangered social parasite of ants. *Proc. R. Soc. Lond. B* 280:20122336. doi: 10.1098/rspb.2012.2336

Thomas, J. A., Elmes, G. W., Wardlaw, J. C., and Woyciechowski, M. (1989). Host specificity among *Maculinea* butterflies in *Myrmica* ant nests. *Oecologia* 79, 452–457. doi: 10.1007/BF00378660

Thomas, J. A., Schönrogge, K., Bonelli, S., Barbero, F., and Balletto, E. (2010). Corruption of ant acoustical signals by mimetic social parasites: *Maculinea* butterflies achieve elevated status in host societies by mimicking the acoustics of queen ants. *Commun. Integr. Biol*. 3, 169–171. doi: 10.4161/cib.3.2.10603

Thomas, J. A., Schönrogge, K., and Elmes, G. W. (2005). "Specializations and Host Associations of Social Parasites of Ants," in *Insect Evolutionary Ecology,* eds M. D. E. Fellowes, G. J Holloway, and J. Rolff (London: Royal Entomological Society), 475–514. doi: 10.1079/9780851998121.0479

Travassos, M. A., and Pierce, N. E. (2000). Acoustics, context and function of vibrational signalling in a lycaenid butterfly–ant mutualism. *Anim. Behav*. 60, 13–26. doi: 10.1006/anbe.1999.1364

Van Dyck, H., Oostermeijer, J. G. B., Talloen, W., Feenstra, V., van der Hidde, A., and Wynhoff, I. (2000). Does the presence of ant nests matter for oviposition to a specialized myrmecophilous *Maculinea* butterfly? *Proc. R. Soc. Lond. B* 267, 861–866. doi: 10.1098/rspb.2000.1082

Van Dyck, H., and Regniers, S. (2010). Egg spreading in the ant-parasitic butterfly, *Maculinea alcon*: from individual behaviour to egg distribution pattern. *Anim. Behav*. 80, 621–627. doi: 10.1016/j.anbehav.2010.06.021

van Zweden, J. S., and d'Ettorre, P. (2010). "Nestmate recognition in social insects and the role of hydrocarbons," in *Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology,* eds G. J. Blomquist and A. G. Bagneres (New York, NY: Cambridge University Press), 222–243. doi: 10.1017/CBO9780511711909.012

Wada, A., Isobe, Y., Yamaguchi, S., Yamaoka, R., and Ozaki, M. (2001). Taste-enhancing effects of glycine on the sweetness of glucose: a gustatory aspect of symbiosis between the ant, *Camponotus japonicus*, and the larvae of the lycaenid butterfly, *Niphanda fusca*. *Chem. Senses*. 26, 983–992. doi: 10.1093/chemse/26.8.983

Wagner, D., and Kurina, L. (1997). The influence of ants and water availability on oviposition behaviour and survivorship of a facultatively ant-tended herbivore. *Ecol. Entomol*. 22, 352–360. doi: 10.1046/j.1365-2311.1997.00077.x

Wardlaw, J., Thomas, J., and Elmes, G. (2000). Do *Maculinea rebeli* caterpillars provide vestigial mutualistic benefits to ants when living as social parasites inside *Myrmica* ant nests? *Entomol. Exp. Appl*. 95, 97–103. doi: 10.1046/j.1570-7458.2000.00646.x

Wasmann, E. (1913). The ants and their guests. *Smithonian Rep*. 1912, 455–474.

Wilson, E. O. (1985). The sociogenesis of insect colonies. *Science* 228, 1489–1495. doi: 10.1126/science.228.4707.1489

Wilson, E. O., and Hölldobler, B. (2005). The rise of the ants: a phylogenetic and ecological explanation. *Proc. Nat. Acad. Sci. U.S.A*. 102, 7411–7414. doi: 10.1073/pnas.0502264102

Witek, M., Barbero, F., and Markó, B. (2014). *Myrmica* ants host highly diverse parasitic communities: from social parasites to microbes. *Ins. Soc*. 61, 307–323. doi: 10.1007/s00040-014-0362-6

Witek, M., Casacci, L. P., Barbero, F., Patricelli, D., Sala, M., Bossi, S., et al. (2013). Interspecific relationships in co-occurring populations of social parasites and their host ants. *Biol. J. Linn. Soc*. 109, 699–709. doi: 10.1111/bij.12074

Witek, M., Sliwinska, E. B., Skorka, P., Nowicki, P., Settele, J., and Woyciechowski, M. (2006). Polymorphic growth in larvae of *Maculinea* butterflies, as an example of biennialism in myrmecophilous insects. *Oecologia* 148, 729–733. doi: 10.1007/s00442-006-0404-5

Wynhoff, I., Bakker, R. B., Oteman, B., Arnaldo, P. S., and van Langevelde, F. (2015). Phengaris (*Maculinea*) alcon butterflies deposit their eggs on tall plants with many large buds in the vicinity of *Myrmica* ants. *Insect Conserv. Divers*. 8, 177–188. doi: 10.1111/icad.12100

Wynhoff, I., Grutters, M., and van Langevelde, F. (2008). Looking for the ants: selection of oviposition sites by two myrmecophilous butterfly species. *Anim. Biol*. 58, 371–388. doi: 10.1163/157075608X383683

Yack, J., Smith, M., and Weatherhead, P. (2001). Caterpillar talk: acoustically mediated territoriality in larval *Lepidoptera*. *Proc. Nat. Acad. Sci. U.S.A*. 98, 11371–11375. doi: 10.1073/pnas.191378898

Yamagushi, S., and Shirozu, T. (1988). *The Life Histories of Five Myrmecophilous Lycaenid Butterflies of Japan*. Tokyo: Kodansha.

Yao, I., and Akimoto, S. I. (2002). Flexibility in the composition and concentration of amino acids in honeydew of the drepanosiphid aphid *Tuberculatus quercicola*. *Ecol. Entomol*. 27, 745–752. doi: 10.1046/j.1365-2311.2002.00455.x

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