MECHANISMS OF COMMUNICATION AND RECOGNITION IN SOCIAL EVOLUTION

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MECHANISMS OF COMMUNICATION AND RECOGNITION IN SOCIAL EVOLUTION

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Editorial: Mechanisms of Communication and Recognition in Social Evolution

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Editorial on the Research Topic

Mechanisms of Communication and Recognition in Social Evolution

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INTRODUCTION

The ability to recognize individuals or classes of individuals and to communicate with fellow group members is crucial for the evolution of complex social behavior. At a minimum, cooperating individuals must convey information that identifies themselves as appropriate partners, and collective behavior requires group members to communicate and synchronize their actions. But how do mechanisms of recognition and communication co-evolve with social behavior, and how do similar signaling abilities arise across animal lineages with vastly different sensory systems and cognitive capacities?

While the mechanisms and sensory modalities of communication have been investigated across diverse animal social systems, progress is uneven across both contexts, and taxonomic divides (Elgar, 2015). Perhaps more significantly, these studies are rarely synthesized to seek common patterns across taxa (but see Rubenstein and Abbot, 2017). The Research Topic *Mechanisms of Communication and Recognition in Social Evolution* takes stock of our current understanding of the proximate mechanisms, selective pressures, and constraints that shape the diversity of communication and recognition systems of social animals, with the intention of inspiring future research directions.

Social animals use signals to communicate information, and an extraordinary diversity of signals have evolved across a range of sensory modalities. Signals may be interpreted in different ways by different individuals or classes of individuals (Stevens, 2013; Wyatt, 2014), and Tumulty and Sheehan argue that understanding the evolution of that diversity requires a shift in the way we think about signals, from considering a single, average receiver to a population of receivers with diverse experiences and motivations. For example, a signal of colony identity may confirm colony membership for some receivers, familiar neighbors for others, and strangers for yet others. Accordingly, Tumulty and Sheehan predict that selection should favor greater uniformity in signals with high receiver agreement, such as for sex recognition, and favor greater signal diversity and flexible learning by receivers for signals where there is low receiver agreement, such as neighbor recognition. The latter prediction suggests that collective learning plays a significant role in determining the level of aggression directed toward non-nestmate neighbors and non-neighbors (e.g., Gill et al., 2012).

IDENTIFYING APPROPRIATE PARTNERS

A common feature of social insects and cooperatively breeding vertebrates is their ability to distinguish between nest- or group affiliates and others, including conspecifics, thereby ensuring that the fruits of cooperative behavior benefit the appropriate recipients (Leonhardt et al., 2016). Nevertheless, the nature and focus of research on this topic varies widely along taxonomic lines. Studies of social insects have focused on the nature of the colony identification signal (typically a cocktail of cuticular lipids and hydrocarbons); the genetic and/or environmental source of between-colony variation in that cocktail; and, most vexingly, the mechanism that allows workers to determine whether the chemical profile of an encountered individual differs from their profile (e.g., Rossi and Derégnaucourt, 2020). In contrast, studies of cooperatively breeding birds have primarily focused on the role of acoustic signals in kin recognition in small family groups (Leedale et al.). Comparatively little is known about group-level or kin recognition cues in social mammals, but the available evidence suggests that olfactory signals, including those produced by the major histocompatibility complex (MHC), play a far more important role in mammals than they do in birds (Brennan and Kendrick, 2006).

Perhaps the most notable difference between social insects and cooperatively breeding vertebrates is that group-level recognition cues appear to be the exception rather than the rule in most vertebrate societies. Although learned group-level vocalizations have been experimentally demonstrated in several cooperatively breeding birds (Sharp et al., 2005; Cockburn et al., 2017) and some mammals (Knörnschild et al., 2012), the capacity to distinguish intruders from group members appears to rely primarily on individual recognition, perhaps because the typically smaller group size allows individual familiarity to play a greater role in recognizing intruders (Riehl and Stern, 2015; Leedale et al.).

Group size may also influence the sensory modality of recognition systems in social insects. It is widely understood that olfaction is the primary sensory modality underpinning nestmate recognition in social insects, a conclusion that may be informed by a taxonomic bias toward ants and termites. However, some social insects, including wasps that form relatively small colonies, use visual cues to distinguish between nestmates and non-nestmates (Hunt and Toth, 2017). Clever experiments reported in Cini et al. reveal an increasing reliance on chemical cues by the wasp *Polistes dominula* with increasing colony size: visual cues are used to distinguish familiar individuals, such as nestmates, from others during the early stages of colony development when colonies are typically small, while older and larger colonies increasingly rely on chemical signals. With larger numbers of individuals within a colony, the capacity to recognize individuals may be impossible, and so workers must recognize classes of individuals. Visual cues may be more efficient for rapidly recognizing a relatively small number of individuals (see Baracchi et al., 2015), since they can accommodate considerable diversity of variants on a common theme (such as facial color pattern), but the extent of this variation may be impractical for chemical signals, where different chemical mixtures may require different receptors. As colony size increases, chemical signals may be more efficient because within-class variation, representing different colony membership or different tasks within a colony, may be less than between-class variation.

Much of the focus of the role of communication in maintaining group cohesion centers around identifying and excluding individuals that are not members of the nest or colony. However, many forms of social behavior require communication to recruit individuals to join groups. Individuals may attract the attention of other group members using food and alarm signals, and individuals of species that form open membership groups may similarly recruit others to a group, if the benefits of increasing group size outweigh the costs (e.g., Elgar, 1986). The capacity to attract conspecifics is critical in the formation of shoals of fish, but these signals may be directed at particular classes of individuals. In an extensive review, Ward et al. document the remarkable preferences of shoal forming fish for particular kinds of associates, based on sex, size, health, relatedness, familiarity, and even color patterns in species with color pattern polymorphism. The benefits to individuals that exercise these preferences for shoal mates have been explored extensively, and fish use a variety of sensory modalities and recognition templates to enable these preferences. The most common recognition template appears to be phenotype matching (see Hauber and Sherman, 2001), which is thought to be learned.

The nests of social insects offer rich pickings for natural enemies, which include a large and taxonomically diverse number of "social parasites" that live within the nest and, in some species, feed on the vulnerable larvae. Given the impressive capacity of social insects to distinguish between nestmates and others, how do these unwanted guests remain safely within the nest of their host? One mechanism is chemical mimicry, where the cuticular chemistry of the guests mimic that of the host, thereby allowing the guests unfettered access to the nest (e.g., Allan et al., 2002). Alternatively, unwanted guests could avoid detection through "chemical insignificance," a term coined by Lenoir et al. (2001) that emphasizes an absence of chemical recognition cues similar to that of just-eclosed callow workers. In their review, Lorenzi and d'Ettorre identify two other ways in which unwanted guests might remain effectively "chemically" insignificant: by ensuring the concentration of cuticular hydrocarbons remains below the discriminator's detection threshold and/or by producing hydrocarbons that are not recognized as cues by the hosts. Rather than erecting new terminology to describe these mechanisms, Lorenzi and d'Ettorre argue it would be more convenient to expand the definition of "chemical insignificance" to incorporate these additional ways in which intruders remain undetected. It is not known whether selection has favored the elimination of detectable odors in these systems, which raises the broader value of using definitions that do not necessarily imply a function. Such "neutral" nomenclature would not exclude consideration of non-adaptive or non-functional explanations, an approach that is emerging for other sensory modalities.

SOCIAL INFORMATION AND APPROPRIATE TASKS

Engaging in social activities self-evidently requires communication, typically in the form of signals that have evolved to alter the behavior of the recipient. This signaling might reflect changes in the environment, e.g., the appearance of predators, or changes in the requirements of the signaller, e.g., by begging for food.

Alarm calls are a conspicuous form of communication in many vertebrates, where individuals vocalize in the presence of predators (Caro, 2005). An ongoing challenge of this research is to demonstrate whether these calls reflect the state of the signaller, or represent functionally referential signals, where the calls refer to particular predators (e.g., Gill and Bierema, 2013; Townsend and Manser, 2013). Nouri and Blumstein show that the nature of alarm calls in marmots are affected by infection with a coccid parasite (although not with infection of several other parasites). Specifically, the calls of infected individuals have higher Wiener entropy values (reflecting higher "randomness" in the sound waves), and so are less precise than those of uninfected individuals. The functional significance, if any, of this variation in alarm calls is unclear because it is not known if or how potential receivers respond. Alarm calls may be used as cues by predators to determine whether the signaller is healthy and thus less easily captured; if so, more vulnerable, infected signalers would be expected to desist or reduce alarm calling, since it increases their risk of predation. On the other hand, alarm calls may also alert conspecifics of potential danger, so the precision of the alarm signal could theoretically provide additional information about its reliability to these receivers. For example, the contact calls of juvenile marmosets have higher Wiener entropy values than those of adults (Takahashi et al., 2017), so the Wiener entropy value of alarm calls could provide information about their reliability if it varies with signaller age.

In social insects, colony efficiency is thought to be improved by workers engaging in specific tasks. The allocation of workers to particular tasks is self-organized, where the collective evaluation of the relative abundance of workers engaged in different activities within the nest relies on individuals being able to recognize the task of nestmates. While several studies confirm that this information is conveyed through chemical signals, typically cuticular hydrocarbons, the nature of these differences is difficult to elucidate because the chemical profile is derived from an extraction of the entire body of the insect. Wang et al. (2016) discovered that the greatest between-colony differences in cuticular hydrocarbons were located on the antennae of meat ants *Iridomyrmex purpureus*, and that workers pay most attention to the antennae of non-nestmates. Following this insight, Wang et al. reveal that task identification signals are located primarily on the legs of workers. Their experiments provide two important insights: that task identification signals are not colony specific; and that cuticular based signaling in social insects is location specific. It remains to be seen whether social insects in general perceive cuticular chemical signals by targeting particular body parts, but this result highlights an important issue—chemical analysis of the cuticular chemical signature of whole-body extracts may be misleading because they can conflate many different signals.

While the honesty of begging signals figures significantly in studies of breeding birds (Mock et al., 2011), little is known about begging honesty in cooperatively breeding species (e.g., MacLeod and Brouwer, 2018), and analyses of provisioning in social insects have largely focused on worker behavior, rather than how larvae signal their nutritional requirements (but see He et al., 2016; Pepiciello et al., 2018). Peignier et al. provide one of the first studies to investigate how genetic factors influence begging by the larvae of ants. In species with higher levels of genetic relatedness, food-deprived ant larvae tend to beg more than their nourished counterparts, but this effect was not apparent in species with low within-colony relatedness, where larvae either did not adjust their behavior or begged more when they were nourished. This study provides a fascinating parallel with recent comparative analyses in birds, which have struggled to find strong correlations between begging honesty and genetic relatedness within the extended family (e.g., Caro et al., 2016; Bebbington and Kingma, 2017). Although begging signals are often unreliable when nest-mates are wholly unrelated to each other—for example, when broods are parasitized by heterospecific nest parasites—the honesty of begging signals does not predictably covary with fine-scale genetic relatedness, probably because it is confounded by variation in the intensity of competition between nest-mates (Bebbington and Kingma, 2017). Peignier et al. nicely circumvent this problem by experimentally manipulating food availability, effectively controlling the extent of competition between larvae. While this study should encourage investigations of social insect larvae as active players in colony life (see also Schultner et al., 2014), it also highlights the importance of considering the intended signal receivers. Varying signaling effort is unlikely to be effective if it does not exact a response: workers did not move significantly more toward the odor of starved larvae than toward the odor of non-starved larvae, perhaps reflecting the lack of differences in cuticular hydrocarbons, alkanes, methylated alkanes, and alkenes between these two groups of larvae. This result contrasts with bumblebees, which reacted more strongly to the odors of food deprived larvae (den Boer and Duchateau, 2006).

The mechanisms of kin discrimination in social birds are thought to involve external cues, often learned during development, which can be used by a receiver as a proxy for genetic relatedness (Leedale et al.). Receivers may infer kinship through familiarity and/or phenotype matching; "genetic" kin recognition is theoretically unlikely and rarely demonstrated empirically (Riehl and Strong, 2015). While familiarity is widely understood to be the primary mechanism that allows kin discrimination, Leedale et al. point out that we have an incomplete understanding of how familiarity allows individuals to recognize kin: what cues are used, what is the sensitive period for association, and how can we distinguish between familiarity and phenotype matching? While vocal cues seem to be the most likely sensory modality, this may reflect an historical research bias, and visual and olfactory cues cannot be ruled out. It is also important to ask whether finely tuned discrimination is adaptive in the context of the behavioral response. When the receiver's decision is binary—e.g., to feed or not to feed a begging nest-mate—the basis of the decision may reflect a threshold rather than quantitative evaluation of the degree of relatedness. Finally, the capacity to discriminate is unlikely where the potential recipients do not vary in relatedness.

Individuals in social groups can be both the source and recipients of information transmitted within the group, which can include the nature and location of potential food, competitors and predators. Individuals may also pay attention to the mating preferences of others within the group, and tend to copy those preferences, a behavior that was first reported roughly 50 years ago (Wiley, 1973; Lill, 1974; Dugatkin, 1992), and subsequently investigated across a broad range of taxa. The meta-analysis by Jones and DuVal reveals that females, and especially inexperienced females, pay attention to the mating behavior of other females, typically preferring to mate with a male if he had been "endorsed" by other females through positive social information. Indeed, observer females were around six times more likely to mate with a generally unpreferred male, if they had seen that male mate with another female. Interestingly, the testing condition (wild or captive) had the strongest impact on mate-choice copying, with females in free-living populations more likely to copy the mate choice of others than females in captive populations. Jones and DuVal identify several possible explanations, but perhaps the most compelling is that females in natural populations are making more genuine choices than those elicited in the typically dichotomous laboratory choices. Clearly, this begs the question of what is the nature of the information observing females acquire, beyond the act of mating—are they learning something about male quality? More generally, it would be interesting to ask whether individuals use social information to make decisions in other contexts, such as responding to rivals and competitors (e.g., Aquiloni et al., 2008; Tibbetts et al., 2020).

PROSPECTS

The nine papers included in this Research Topic illustrate and build on many of the major advances in the field of social animal communication over the past several decades, from conceptual developments in signal honesty and evolution (e.g., Peignier et al.) to the technical innovations that allow human researchers to eavesdrop on the chemosensory modalities of their study animals (e.g., Wang et al.; Lorenzi and d'Ettore). There is an increasing realization that common selective pressures and constraints may underlie the evolution of communication mechanisms in seemingly disparate contexts (for example, mate choice, kin discrimination, and intraspecific competition; Jones and DuVal; Tumulty and Sheehan; Leedale et al.) and that seeking parallels across contexts and taxa may yield the greatest insights into these evolutionary processes. However, this Research Topic also highlights the many gaps remaining in our knowledge and raises new challenges for future research.

Many questions remain about the cognitive mechanisms that enable recognition cues to be learned. For example, phenotype matching is thought to be widely used to identify membership of particular classes of individuals, including potential mating partners (e.g., Kopp et al., 2018); neighbors (Crepy and Casal, 2015, but see Till-Bottraud and de Villemereuil, 2016); offspring (e.g., Yang et al., 2019); and social groups (Ward et al.). Phenotype matching is especially important for social species, to direct cooperative behavior toward particular individuals (typically group or colony mates; Leedale et al.; Rossi and Derégnaucourt, 2020) or to detect extra-group parasites (Shizuka and Lyon, 2010). Phenotype matching occurs when the individual references its own traits, often referred to as a template, against that of another individual, and responds accordingly (Hauber and Sherman, 2001; Mateo, 2004). The template comprises signals (or cues) that may include odors, sounds or color patterns. While there is evidence for such templates (e.g., Rossi and Derégnaucourt, 2020), very little is known about how individuals learn a self-referencing template. For vertebrates, the challenge is to distinguish whether recognition results from familiarity or phenotype matching, to determine the extent to which self-referential templates might be genetically determined, and to identify developmentally sensitive periods in which such templates might be learned (Leedale et al.). For social insects that rely primarily on chemical cues found on the insect cuticle (but see Cini et al.), the challenge lies in accounting for the many factors that may change the nature of these chemicals, including diet, ontogeny, and task (Henneken et al., 2017; Otte et al., 2018; Wang et al.); and that a complete absence of cues is an effective mechanism of by-passing this recognition mechanism (Lorenzi and d'Ettorre). Perhaps familiarity plays an important role in recognition cues in social insects (e.g., Gill et al., 2012).

Another challenge is to understand how the physiology and morphology of sensory systems of receivers have coevolved with social signals. In social mammals, neurological structures associated with olfactory learning and recognition of individuals have coevolved with the expression of odorant molecules, and chemo signals that likely originated in the context of mating and parental care have been co-opted for use in cooperative behaviors (for example, in kin recognition; Brennan and Kendrick, 2006). More recent evidence in social insects suggests similar co-evolutionary patterns between social behavior and receptor organ morphology (Wittwer et al., 2017), and evolutionary links between social behavior, signals, learning, and perception may emerge in other systems and species (Miller et al., 2020). Vocal repertoire size in primates is correlated with group size (McComb and Semple, 2005), but in birds has increased with the evolution of cooperative breeding, but not group size or group stability (Leighton, 2017). The associated neurological requirements for processing this information have led to hypothesized links between social complexity and brain size, with mixed empirical support across taxa (Godfrey and Gronenberg, 2019). Does social complexity, and its attendant requirements for sophisticated communication and individual recognition, drive signal evolution? Or do pre-existing coevolved sensory systems between signalers and receivers allow societies to increase in complexity (Freeberg et al., 2012)? While it is self-evident that communication underpins social behavior, unpicking these details continues to offer intriguing research opportunities.

AUTHOR CONTRIBUTIONS

ME and CR conceived, wrote, and edited the manuscript, and approved the submitted version.

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Parasites Are Associated With Noisy Alarm Calls

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Many animal signals used for mate choice assessment are condition dependent, but less is known about the condition dependence of other biologically important signals. We asked whether yellow-bellied marmot (*Marmota flaviventer*) alarm calls varied as a function of parasite infection and/or neutrophil:lymphocyte ratios (a measure of immunological challenge). We found that marmots infected with *Eimeria*, an intestinal parasite, had noisier calls. This is potentially because of an immunostimulating effect of *Eimeria* infection which may draw energy from nonvital functions. The results suggest calls potentially contain information about parasite status which could be used by receivers to estimate a caller's condition. Future studies are required to determine whether infection influences caller reliability and how receivers respond to alarm calls from parasitized individuals.

Keywords: *Marmota flaviventer*, alarm calls, communication, health, parasites

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INTRODUCTION

Many animal signals such as skin or feather color and vocalizations are condition dependent, which means that signal expression varies as a function of an individual's health or nutritional status (Appleby and Redpath, 1997; Von Schantz et al., 1999; Møller et al., 2000; Scheuber et al., 2003). Much of the literature on condition-dependent morphological traits is focused on mate choice. For instance, the large literature on carotenoids and guppy (*Poecilia reticulata*) coloration shows that bright skin color is sexually selected (Kodric-Brown, 1989) because it is an indication of health (Grether et al., 2004) and/or foraging ability (Grether et al., 2001). This is the case because carotenoids are a limiting factor in pigment production and are relatively scarce in the environment (Grether et al., 1999); animals with bright skin coloration have consumed more carotenoids than dull males and may therefore be better at foraging (Grether et al., 2001). This relationship between physical or physiological condition and signal structure extends to acoustic signals as well.

In many species, acoustic structure of vocal signals can vary based on state-dependent factors such as age (Simmons and Zuk, 1992), the amount of energy reserves within muscles (Bevier, 1997), body size and mass (Appleby and Redpath, 1997), hormone levels (Fusani et al., 1994; Marler and Ryan, 1996), and nutritional state (Noguera et al., 2010). In fact, the acoustic variation in these vocal signals are often used by conspecifics to select healthy mates (Fusani et al., 1994; Beani and Dessi-Fulgheri, 1995) which suggests vocal signals are an indicator of health and physiological condition. Furthermore, Appleby and Redpath (1997) also suggested that calls can be costly to emit and may therefore be an honest indicator of physiological condition.

The present study focuses specifically on the potential information content of acoustic signals as indicators of health status. Immune system activation, a common indicator of health or disease has been associated with increases in the inter-pulse interval of cricket (*Gryllus bimaculatus*) songs

(Fedorka and Mousseau, 2006), and parasitic infections have been associated both with fewer terminal notes in white-crowned sparrow (*Zonotrichia leucophrys oriantha*) songs (Munoz et al., 2010) and with increases in note frequency and decreases in length of calls in tawny owls (*Strix aluco*) (Appleby and Redpath, 1997). Because crickets (*Gryllus campestris*) increase the energetic investment in their stridulatory signals with increased food availability (Scheuber et al., 2003), one explanation for these changes in the structure of acoustic signals is that they are sensitive to anything that influences energy allocation (such as disease or body condition). Similarly, sparrows infected with avian malaria, which effects the ability of their blood to bind to oxygen, had fewer terminal notes, again providing a concrete mechanism linking infection status to vocal output.

The acoustic structure of alarm calls and other vocalizations produced when animals are stressed may be influenced by both external factors (e.g., predator type, degree of risk, etc.), internal factors (physiology, health status), and individual factors (e.g., anatomical differences). For instance, in dogs (*Canis lupus familiaris*), individuals which were recently unhealthy (defined by recent treatment in a veterinary clinic) emitted barks in which the harmonic to noise ratios were significantly higher than in the calls of healthy dogs (Riede et al., 2001), and in pigs (*Sus scrofa*), increased pain and stress resulted in calls that were more clear and piercing (Puppe et al., 2005). More specifically, physiological stress–which may result from infections–can affect the Weiner entropy of alarm calls. For instance, as fecal glucocorticoid levels increased, yellow-bellied marmot (*Marmota flaviventer*) alarm calls became less noisy (as measured by Weiner entropy) (Blumstein and Chi, 2012). While the effects vary, all of these previous studies provide evidence for the association between physiological stress and/or condition and the structure of vocalizations produced by stressed animals. Such relationships are not entirely unexpected as infection and mounting an immunological response may reduce energy to allocate to vocalizations (Scheuber et al., 2003; Fedorka and Mousseau, 2006) and this may potentially influence the structure of alarm calls.

While any vocalization may be described by a variety of acoustic measurements, we focused here on changes in Weiner entropy of alarm calls. These calls are emitted when animals encounter predators; a fear-inducing situation. Prior work has shown that deterministic chaos or noise (which was used to simulate deterministic chaos), and other acoustic non-linearities (e.g., subharmonics, biphonation, rapid frequency shifts, and rapid amplitude shifts) are associated with fear in humans and animals (Blumstein and Recapet, 2009; Blumstein et al., 2010, 2012; Townsend and Manser, 2011; Slaughter et al., 2013; Blesdoe and Blumstein, 2014), and changes in Weiner entropy are associated with fear-driven stressors (Blumstein and Chi, 2012).

Given that our understanding of the relationship between physiological condition and the structure of alarm vocalizations is in its infancy, we asked whether call noisiness (which we measured by Weiner entropy) is associated with: (1) the presence of specific intestinal parasites; (2) total intestinal parasite diversity; and (3) marmots' neutrophil:lymphocyte ratio. Prior work has shown that call structure is influenced by intestinal parasite load in tawny owls (Redpath et al., 2000) and that specific parasites differentially effect vocal structure in white-crowned sparrows, as described above (Gilman et al., 2007). Thus, we expected that parasite infection would modify marmot alarm call structure but the precise way that it would was an open question. Additionally, because the energy required to mount an immune response (measured by N:L ratios) may cause energy to be reallocated toward improving health rather than defense or signal production (Scheuber et al., 2003; Fedorka and Mousseau, 2006), we expected that there would be a relationship between call structure and N:L ratio as well.

If the nonlinearity and fear hypothesis explained call noisiness, then we would expect that individual parasites, an increase in parasite diversity, and increased N:L ratios would be associated with increased noisiness. Interestingly, prior work showed that marmots with higher baseline glucocorticoid levels produced calls with reduced, rather than increased, Weiner entropy (Blumstein and Chi, 2012) suggesting that more arousable individuals may siphon energy away from call mechanics. Thus, the question of whether factors that influence perceived vulnerability or risk are associated with increased noisiness remains unresolved.

MATERIALS AND METHODS

Subjects and Data Collection

We studied a wild population of yellow-bellied marmots in and around the Rocky Mountain Biological Laboratory during their active seasons from 2003 to 2017. We aimed to live-trap marmots every other week during the snow-free part of their active season (May–September) during which animals were marked (ear tags for permanent identification and fur dye that lasted until their next moult) and a number of physiological samples were collected.

If marmots produced alarm calls when trapped, we recorded these human-elicited alarm calls using an Audix OM-3xb microphone (frequency response: 40 Hz—20 kHz) located 20–40 cm from the trap. Prior work has shown that yellow-bellied marmot calls communicate risk, not predator type (Blumstein and Armitage, 1997). Thus, by standardizing the context of call production, we could focus on physiological correlates of call structure. The calls were recorded onto either a Sony PCM-M1 digital audio tape recorder or a Marantz PMD 660 direct to disk recorder. All samples were saved at 44.1 kHz and 16 bit resolution. Recordings with noticeable background noise or calls that were clipped were removed from subsequent analyses. Each call was edited into a 1 s file for subsequent analysis in Sound Analysis Pro (Ofer Tchernichovski, City College of New York). Calls are generally much shorter than 1 s; thus 1 s was chosen to standardize the measurements. This sound clip always contained the entire call. Sound Analysis Pro calculates Wiener entropy. Wiener entropy ranges from 0 (pure noise) to negative values that indicate increasing structure (or decreased entropy). Thus, noisier calls had values closer to 0.

As part of the routine live-trapping between 2003 and 2008, we also collected fecal samples from marmots that defecated in the trap or while we were processing them for a subsequent study of intestinal parasites. The sample was stored in formalin immediately. Within 6 months, fecal samples were analyzed by performing fecal floats using Ova FloatTM Zn 118 (zinc sulfate heptahydrate; Butler Animal Health Supply, Dublin, OH, USA). Up to one sample was used for each individual in a given month. The wet slides were then scored for presence of three fecal-orally transmitted (MacNeal, 1904) intestinal parasites: *Ascaris* (a nematode), *Eimeria* (a coccidian), and *Entamoeba* (a protozoan) (Lopez et al., 2013). Intestinal parasites have been associated with decreased food intake and anorexia (Jones et al., 2006; Laurenson et al., 2011) and in marmots, *Ascaris* has been associated with less time spent foraging (Chmura et al., 2016).

Finally, also as part of routine live-trapping, we collected up to a 2 ml blood sample from the femoral vein of the marmots, placed it in a heparin-filled tube, and made a thin film blood smear within 2 hours of collection (Chmura et al., 2016). Slides were stained using the Hema 3 Stat Pack (Thermo Fisher Scientific, Inc., Waltham, MA, USA) (Wey and Blumstein, 2012). We used a standard procedure to calculate the neutrophil:lymphocyte (N:L) ratio where we counted neutrophils, lymphocytes, basophils, and monocytes up to 100 cells or for 30 min (whichever came first). *Trypanosoma* (which may be transmitted by fleas; MacNeal, 1904) presence was a binary measure—either present or not—and was noted during the white blood cell counting. *Trypanosoma* has been associated with more time spent foraging and less time spent vigilant in marmots (Chmura et al., 2016).

There are a number of quantifiable traits associated with health status. We used neutrophil:lymphocyte (N:L) ratios as an indicator of changes in health. Prior work has found that increases in N:L ratios are associated with continuously high levels of glucocorticoids indicating chronic stress (Swan and Hickman, 2014). Changes in N:L ratios have also been associated with injury and infection in koalas (*Phascolarctos cinereus*, Bolliger and Backhouse, 1960) and neutrophils play a critical role in mounting an immunological defense against systemic infections in mice (Conlan, 1997). Higher N:L ratios can therefore indicate the immunological challenge of certain infections or in this case, can indicate activation of an immune response and allocation of energy away from antipredator defenses.

Only alarm calls with either an associated blood sample or fecal parasite data (defined as collected within 10 days of the call) were used in the study. The sample sizes were therefore different for the alarm call to N:L ratio and alarm call to *Trypanosoma* presence as opposed to the analysis of the alarm calls to intestinal parasites. The sample size for the N:L ratio and *Trypanosoma* presence consisted of 836 calls from 107 individuals whereas the sample size for the fecal parasite presence consisted of 536 calls from 62 individuals. In total (both blood and fecal analyses) 146 individuals were represented (70 males and 76 females) for which we had 3-5 calls from each individual recorded at a given recording session.

Statistical Analysis

We fitted a series of linear mixed effects models using lme4 (Bates and Maechler, 2018) and lmerTest (Kuznetsova et al., 2018) in R (R Development Core Team, 2009) to explain variation in Wiener entropy. Fixed effects included age, sex, and one of the measures of condition—neutrophil:lymphocyte ratio, the presence of *Trypanosoma*, the presence of *Ascaris*, *Eimeria*, *Entamoeba*, or the total number of species of fecal parasites (i.e., the sum of *Ascaris*, *Eimeria*, and *Entamoeba*). Marmot identity was fitted as a random effect. We examined residuals to confirm the models were appropriate for the data structure.

Ethics

Marmots were studied under annual permits issued by the Colorado Division of Wildlife (TR-917). All procedures were approved under research protocol ARC 2001-191-01 by the University of California Los Angeles Animal Care Committee on May 13, 2002, and renewed annually.

RESULTS

After controlling for significant age effects ($p < 0.001$) and non-significant sex effects ($p = 0.514$ for N:L ratio and 0.523 for *Trypanosoma*), neither the neutrophil to lymphocyte ratio ($p = 0.925$) nor the presence of *Trypanosoma* ($p = 0.568$) had a significant effect on Wiener entropy (**Tables 1A,B**). We did however find a significant effect of *Eimeria* on marmot alarm calls ($p < 0.001$) such that animals infected with *Eimeria* produced noisier calls with higher Wiener entropy values (**Table 1C**). Similarly, animals with more intestinal parasites in general produced calls with higher Wiener entropy ($p = 0.008$) (**Table 1D**). After adjusting for age and sex, we found no significant effects of *Ascaris* ($p = 0.708$) or *Entamoeba* ($p = 0.231$) on the noisiness of marmot alarm calls (**Table 1C**).

DISCUSSION

Despite a growing literature that shows a relationship between vocal structure and disease or health status (Appleby and Redpath, 1997; Fedorka and Mousseau, 2006; Munoz et al., 2010; Noguera et al., 2010), the Weiner entropy of yellow-bellied marmot alarm calls was not significantly associated with the presence of *Ascaris*, *Entamoeba*, or *Trypanosoma*, or by an individual's neutrophil:lymphocyte ratio. Alarm calls were, however, significantly noisier for marmots infected with *Eimeria* and for marmots with higher fecal parasite diversity.

These results could reflect how each parasite stimulates or suppresses the immune system. Yun et al. (2000) noted that many *Eimeria* oocysts are required to generate an immune response, and we note that it was not difficult to detect *Eimeria*—marmots shed many oocysts. By contrast, many parasites have immunosuppressive effects and this includes *Ascaris* (Faquim-Mauro and Macedo, 1998), *Entamoeba* (Soboslay et al., 2006; Lejeune et al., 2009), and *Trypanosoma* (Hirokawa et al., 1981; Albright et al., 1990). The changes we saw in the alarm calls may be explained by *Eimeria* stimulating an immune response such that individuals divert energy away from vocalizations and toward immune response and therefore may not have sufficient energy to enable them to produce "proper" calls. By contrast, marmots infected with an immunosuppressive parasite may not divert energy and hence could maintain the ability to emit proper

TABLE 1 | Results from linear mixed effects models explaining variation in alarm call entropy as a function of: **(A)** N:L ratio, **(B)** the presence or absence of *Trypanosoma* sp., **(C)** the presence or absence of each individual intestinal parasite, and **(D)** intestinal parasite diversity.

(A)	**Variable**	**Estimate**	***SE***	***df***	***t***	***P*-value**
Fixed effects						
	N:L ratio	−0.005	0.055	817.519	−0.094	0.925
	Sex	0.197	0.300	104.121	0.655	0.514
	Age	0.312	0.018	804.307	17.280	<0.001
	Variable	**Variance**	***SD***			
Random effects						
	Individual id	2.288	1.513			
	Residual	0.524	0.724			
(B)	**Variable**	**Estimate**	***SE***	***df***	***t***	***P*-value**
Fixed effects						
	Trypanosoma	0.122	0.217	843.541	0.571	0.568
	Sex	0.192	0.299	103.867	0.641	0.523
	Age	0.313	0.018	799.029	17.446	<0.001
	Variable	**Variance**	***SD***			
Random effects						
	Individual id	2.271	1.507			
	Residual	0.524	0.724			
(C)	**Variable**	**Estimate**	***SE***	***df***	***t***	***P*-value**
Fixed effects						
	Ascaris	−0.056	0.148	509.022	−0.375	0.708
	Eimeria	1.098	0.189	528.777	5.802	<0.001
	Entamoeba	−0.203	0.169	524.054	−1.200	0.231
	Sex	−0.069	0.218	60.055	−0.316	0.753
	Age	0.104	0.034	206.007	3.040	0.003
	Variable	**Variance**	***SD***			
Random effects						
	Individual id	0.600	0.775			
	Residual	0.531	0.729			
(D)	**Variable**	**Estimate**	***SE***	***df***	***t***	***P*-value**
Fixed effects						
	Total parasites	0.243	0.092	502.546	2.646	0.008
	Sex	−0.075	0.218	59.968	−0.344	0.013
	Age	0.086	0.034	196.655	2.518	0.008
	Variable	**Variance**	***SD***			
Random effects						
	Individual id	0.599	0.774			
	Residual	0.560	0.748			

For each fixed effect, model estimates, SE, standard error; df, degrees of freedom, t-value, and p-value are displayed. N:L ratio stands for neutrophil to lymphocyte ratio. For each random effect, variance and standard deviation (SD) are included.

calls. If this is the case, call acoustics is indeed an indicator of the caller's condition. Alternatively, infected individuals are more vulnerable and hence produce higher-risk calls (see below). Whether this potential information is used by receivers remains an open question.

Both conspecifics and predators can use information contained in calls and it is profitable to view a potential communication system from both perspectives. From the conspecific's perspective, a sick caller may be less reliable if they are more vulnerable than normal and this enhanced vulnerability is communicated acoustically. Rodents are indeed capable of associating individuals with reliability (Hare and Atkins, 2001; Blumstein et al., 2004). However, prior work has shown that physiological stress is associated with calls with *less* Weiner entropy—scared marmots articulate their calls (Blumstein and Chi, 2012). If the valence of entropy is such that more scared animals produce less noisy calls, then these results suggest that *Eimeria* influences how marmots perceive risk; infected marmots perceive less risk, not more risk. Some parasites with complex multi-host lifecycles are known to influence risk assessment (e.g., *Toxoplasma gondii*, Berdoy et al., 2000), but by doing so those parasites increase the likelihood that they reach their next host. This is not the case with *Eimeria* which reproduces inside marmots. Future studies should look for associations between *Eimeria* infection and other antipredator traits (e.g., maximum running speed). Regardless of the mechanism, this information about risk perception may translate to a less reliable caller resulting in perceivers acting less vigilant or habituating to the calls from sick individuals (Hare and Atkins, 2001).

From, a predator's perspective a sick caller might be more attractive in that it may be less able to defend itself. Prior work has found that less popular or more docile marmots are more likely to call (Fuong et al., 2015) and that altering these calls may be due to an increased vulnerability from being in a smaller group or otherwise more socially isolated (Fuong et al., 2015). Sick marmots may also be less able to defend themselves and therefore more vulnerable. If marmots do indeed alter their call structure when they are more vulnerable, this could explain the difference in entropy of marmots infected with *Eimeria*. By signaling their vulnerability, sick marmots could conceivably solicit help from others—something that we have not obviously noticed in the field, but nevertheless requires more detailed study to properly reject.

While the potential consequences of the results are speculative, these results combined with prior results from a variety of species, suggest that internal state, including parasite status, may generally influence the structure of a variety of vocalizations, including alarm vocalizations. From an applied perspective, such information could be used to non-invasively monitor an individual's health status. Future work determining whether and how marmots respond to the calls from healthy and sick individuals would shed more light on whether, and how, this potential information is used. Additionally, studying the underlying affects of immune system response on vocalizations may help determine which vocalizations suggest disease or other health information in wild populations.

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

DB conceived idea, collected field data, and guided analyses. KN measured alarm calls and helped analyze data. Both authors wrote the manuscript.

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Task-Specific Recognition Signals Are Located on the Legs in a Social Insect

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Task allocation ensures a high level of organization within social insect colonies. Workers reveal their task assignment through cuticular hydrocarbon (CHC) signals. The source and chemical composition of these signals are largely unknown. We ask whether task recognition signals are located on particular body parts of workers of Australian meat ants (*Iridomyrmex purpureus*). We analyzed the CHC profile on the antennae, legs, and abdomens of workers engaged in different tasks. Discriminant analysis showed that the leg profile is the best indicator of task identification. Behavioral assays confirmed this finding: workers typically reacted differently to non-nestmates engaged in different tasks, but not if the CHCs on the legs of their opponents were removed by a solvent. Lasso and Elastic-Net Regularized Generalized Linear Model (GLMNET) revealed which CHC components show the highest correlation in task and nestmate recognition, suggesting that social insects can simultaneously convey different CHC signals on different body parts, thereby allowing efficient signaling and signal perception.

Keywords: social insects, task recognition, cuticular hydrocarbons, body parts, GLMNET

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INTRODUCTION

Social insect colonies are self-organized, with social cohesion achieved by individual workers making simple decisions based on their interactions with other workers (Sachs, 2004). A well-developed task discrimination signal system is crucial because it allows workers to determine the tasks of others, assess the needs of the colony (Gordon and Mehdiabadi, 1999; Sachs, 2004) and respond accordingly (Wagner et al., 1998; Gordon and Mehdiabadi, 1999). Understanding how the labor force is distributed in social insect colonies therefore requires some knowledge of the nature and source of these signals (Anderson and McShea, 2001). For example, the mechanism of reproductive tasks signaled by queen mandibular pheromone is well understood because the chemical components and their site of synthesis in mandibular glands are clearly defined, allowing experimental manipulation of behavior using synthetic chemicals (Dietemann et al., 2003; Strauss et al., 2008; Kocher and Grozinger, 2011; Nunes et al., 2014).

Cuticular hydrocarbons (CHCs) are the most abundant chemicals on the surface of ants, and play key roles in several communication contexts, including nestmate recognition (Martin et al., 2008; van Wilgenburg et al., 2010), task recognition (Wagner et al., 1998; Greene and Gordon, 2003; Nascimento et al., 2013), trail marking (Hölldobler et al., 2004), recruitment of foragers (Greene and Gordon, 2003), and queen viability and fertility signals (Cuvillier-Hot et al., 2004; Lommelen et al., 2006; van Oystaeyen et al., 2014). The role of CHCs in communicating tasks is poorly understood.

The main challenge to understanding these signals is to identify their chemical components. This requires establishing a bioassay that provides a consistent measure of signal perception; identifying the source of the signal; and synthesizing the putative signaling hydrocarbons. In ants, the level of aggression provides a precise measure of task perception, because workers engaged in different tasks not only exhibit different levels of aggression toward non-nestmates (Sturgis and Gordon, 2013), but also elicit different levels of aggression from non-nestmates (Nascimento et al., 2013). Typically, studies of chemical signals use coupled gas chromatography mass spectrometry (GCMS) to identify the CHC profile obtained from the entire body of the insect: the "whole body" profiles of individuals engaged in different tasks are then compared using principal components and/or discriminant analyses (Heinze et al., 2002; van Wilgenburg et al., 2006; Lenoir et al., 2009; Martin and Drijfhout, 2009b). While these studies reveal broad differences in CHC profiles across different tasks (Wagner et al., 1998; Kaib et al., 2000; Nascimento et al., 2013; Sturgis and Gordon, 2013), and that cocktails of CHCs elicit different behaviors (Wagner et al., 1998; Greene and Gordon, 2003), the identity of the chemicals responsible for signaling different tasks remain unknown. The list of putative chemicals may be reduced substantially if signals indicating task activities, like colony identification signals, are located on specific body parts (see Wang et al., 2016).

Like many ants, task allocation in the Australian meat ant *Iridomyrmex purpureus* is largely determined by an age polyethism. Newly emerged workers usually tend the brood inside the nest, gradually switching to tasks outside the nest, including maintaining the nest surface (nest maintainers), foraging for food (foragers), and engaging in ritualized display behavior (displayers) with neighboring colonies (van Wilgenburg et al., 2005). Ants can also respond to changes in the ambient environment, allowing individuals to adjust their behavior according to the specific needs of the colony (Gordon and Mehdiabadi, 1999). For example, foragers and nest maintainers may form a line of defense by displaying in large numbers when the colony is under siege from neighbors. The ritualized display behavior is unambiguous, and forms part of a repertoire of increasingly aggressive behaviors directed toward non-nestmates (van Wilgenburg et al., 2005, 2006). Display does not usually escalate into other aggressive behavior, such as biting or fighting and rarely results in injury or death.

Following Wang et al. (2016), who showed that workers of *I. pupureus* paid more attention to the antennae of non-nestmates and the legs of nest-mate workers, we investigate the role of location specific CHCs in signaling task allocation in *I. purpureus*. Specifically, we compare the CHC profile across different body parts of workers engaged in different tasks. We use behavioral assays to identify which body part profiles are most informative of task activity, and experimentally washed chemicals from the putative body part to confirm the role of that profile. Finally, we use GLMNET (Friedman et al., 2009) to identify the variation in CHC components that correlate best with the variation in task discrimination and nestmate recognition signals. GLMNET is a fast and reliable method of selecting variables, and resolves the problem where the number of chemical components is larger than the number of samples, a common issue for studies of complex chemical signal mixtures.

MATERIALS AND METHODS

Collection of Workers

We sourced Australian meat ants (*Iridomyrmex purpureus*) from colonies located in Serendip Sanctuary, 60 km south-west of Melbourne, Victoria, Australia. All samples were collected between January and March 2015. For chemical analyses and behavior assays, workers of different tasks were collected from 12 distant mature colonies (six colony pairs, colonies of each pair were at least 300 meters apart) while they were performing specific tasks, kept temporarily in clean glass containers, and used within 1 h after collecting to avoid any changes in surface signals. Nest maintainers were collected when they were carrying or moving pebbles on the surface of the nests; foragers were collected from returning workers on foraging trails with food (insect parts) in their mandibles. The displayers were collected where workers were engaged in ritualized display behavior. To avoid collecting ants from the neighboring colonies, each displayer was placed with four workers from the home colony, and only individuals showing no antagonistic behavior were included.

Cuticular Hydrocarbon (CHC) Analysis

The chemical components were identified and confirmed with gas chromatography-mass spectrometry (GC-MS) following Wang et al. (2016). We compared the colony and task CHC profile variation of different body parts by collecting 15 ants (five individuals of each of three tasks) from each of four colonies ($n = 60$). These individuals were freeze-immobilized within 20 min of collection, and their antennae, heads, legs, and abdomens were dissected with a pair of surgical scissors. Individual body parts were immersed in 200 μL hexane (HPLC grade) for 10 min to extract CHCs, dried under a nitrogen flow and redissolved with 10 μL hexane including tridecane (100 mg/L) as an internal standard. To analyse the abundance of each component in the signals, 1 μL samples were injected into an Agilent 7820A gas-chromatograph with a split/splitless injector and flame ionization detector (GC–FID) and a Zebron ZB-5 capillary column (Phenomenex, USA; 30 m, 0.25 mm in diameter, 0.25 μm film thickness). The oven temperature was raised from 75 to 260°C at 10°C/min, then raised to 325°C at 5°C/min and held for the last 5 min. The injection temperature was 250°C, and detector temperature was 325°C. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. The Agilent ChemStation (E. 02.02) was used to acquire the GC-FID data, and peak area was integrated using the default setting. We minimized the effects of contamination by including only those peaks that exceed 0.1% of the total peak area, and had a peak area larger than 20 μV/s in the analysis. Peak area is usually much larger than 20 μV/s, and anything smaller could not be distinguished reliably from the baseline. As a result, 24 peaks were identified in the samples of antennae and 47 peaks in the leg and abdomen samples. The identification of CHC components follows a previous study on this species (van Wilgenburg et al., 2006), but GC-MS was also used to confirm each component. The GC-MS analysis was carried out on an Agilent 5975C quadrupole mass spectrometer coupled directly with an Agilent 7890A GC and fitted with a Zebron ZB-5MS column. The MS transfer line was set at 280°C, the ion source adjusted to 250°C and the quadrupole operated at 150°C, with the oven program the same as the GC-FID. Helium was used as the carrier gas at a flow rate of 0.8 ml/min.

Task Determination by Body Size

After hexane extraction, the heads of the workers were preserved for measurements. We measured the width of the heads as body length and body weight vary with the different nutrient history of each individual (van Wilgenburg et al., 2005). Workers of *I. purpureus* can store nectar in their crops, which could affect their body length and weight. The heads of the individuals were placed on an Epson 7520 scanner, and scanned with a reference scale. The width of each head was then measured from the scanned picture with *Nano Measurer 1.2*. A total of 257 ants of the three tasks from seven colonies were analyzed.

Behavioral Assays

Discrimination of Tasks

To test whether workers of *I. purpureus* can discriminate the tasks of other workers, we conducted two-on-two aggression assays in containers (7 cm by 10 cm) lined with fluon (Dreier et al., 2007). The assays took place between 10 a.m. and 4 p.m., when the ants are most active. We collected workers from 12 colonies, which were assigned into six colony pairs. For each pair of colonies, foragers from one colony were selected as focal ants because their task and colony origins were easy to identify and they were likely to have encountered different tasks of other nests. The ants were marked individually on the dorsal surface of their thorax with small dots of enamel paint, and allowed to acclimatize in a holding container for at least 5 min. The two focal workers were then placed in the assay container, and two unmarked nest maintainers, foragers, or displayers, were then gently placed into the container. We recorded the behavior of the marked, focal ants toward the stimulus ants, and the behavior of the stimulus ants toward the focal ants for 3 min, using a Panasonic HDC-SD80 video camera. The video was then replayed in slow motion, allowing us to record the number of antennations of the focal individuals toward different body parts of their opponents and the duration of the display behavior. Following Wang et al. (2016), we included antennations toward the antennae and legs only, because we rarely saw antennation toward the thorax, which in any case had been marked with identifying paint. We recorded the behavior of 108 focal workers and 108 stimulus workers, each comprising three trials (six individuals) for each of the three combinations (focal foragers with stimulus nest maintainers, foragers, and displayers), for each of the six colony pairs. We measured the response to non-nestmates because workers of *I. purpureus* rarely respond aggressively toward nestmates. The behavior was recorded and observed blind to the task and colony source of the opposing ants.

Location of Task Specific Signals

To test whether the CHCs on the legs of *I. purpureus* convey task specific signals, we removed the signals on their legs using a method similar to that published previously (Wang et al., 2016). Frontal femurs of ants of both treatments were amputated to prevent them from grooming, which might restore the chemical signals on legs and antennae (Lucas et al., 2004; Boroczky et al., 2013). After freeze anesthetization at −20°C for 3 min, either one side or both sides of the mid and hind legs of the experimental workers were carefully immersed in cold hexane in a small glass vial for at least 1 min to remove most CHCs from the legs. Hexane was always carefully kept away from direct contact with the abdomen or thorax of ants, as this could have killed them. Ants with signals from one side removed were used as controls for the manipulation and the impact of the hexane on the mobility of ants. After the operation, ants were allowed to recover in clean containers for at least 15 min before behavior assays, and only individuals that showed no obvious loss of mobility were selected for behavior assays. We placed two marked, focal foragers from one colony of each colony pair ($n = 5$ pairs of colonies) into the assay container with two manipulated nest maintainers, foragers, or displayers from the other colony, and recorded the behavior of the marked, focal ants for 2 min. Each of the two treatments was replicated four times between five colony pairs and three combinations ($n = 240$). 92.5% (37 out of 40 individuals) of the remaining hexane treated ants were alive 24 h after the tests, and 95.0% (38 out of 40 individuals) of the untreated ants survived after the tests, indicating that our protocols did not significantly reduce the viability of ants during the test. The antennation behavior and the duration of display behavior were recorded with the observer blind to the colony source of the opposing ants and/or the treatment.

Statistical Analyses

Task Discrimination by Body Size

Differences in body size between colonies or task groups were investigated using least squares ANOVA with colony membership and task groups as main effects. These analyses were conducted using JMP version 9 (SAS Institute).

Chemical Analyses

The relative proportions of each quantifiable peak area to that of the total sample were calculated using the following formula: Proportion of Area $= A_i/\sum A_n$, where A_i = area of peak i, and $\sum A_n$ = total peak area, and these transformed peak areas were used for subsequent analyses. We used principal components analysis (PCA) with the transformed peak areas to reduce the number of describing variables for a discriminant function analysis (DA). We extracted the resulting components with eigenvalues which together explained at least 85% of the total variance. We then performed discriminant analysis with the scores produced by the PCA to investigate variation between body-part specific profiles, and whether colonies or tasks could be separated on the basis of the hydrocarbon profiles of different body parts. PCA and DA were conducted using JMP version 9 (SAS Institute). Unless stated otherwise, values are means ± SE.

Behavioral Analyses

Differences in the frequency of antennation and the duration of display behavior in the task discrimination test were analyzed separately for the focal workers and for the stimulus workers, using mixed effects models with task (displayer, forager, or nest maintainer) as a main effect and colony identity as a random effect, with the variance partitioned using restricted maximum likelihood (REML). We used a mixed effects model to investigate how experimental manipulation of signals located on the legs influenced the duration of display behavior, using task (displayer, forager, or nest maintainer), and treatment (three vs. six legs washed) as main effects and colony identity as a random effect, with the variance partitioned using REML. Where appropriate, we used *post-hoc t*-tests. All of these analyses were conducted using JMP version 13 (SAS Institute).

Signaling Components

Generalized Linear Modeling is an extension of regression models designed to deal with error distributions beyond the normal distribution. Lasso and Elastic-Net Regularized Generalized Linear Model (GLMNET) fits a Generalized Linear Model via penalized maximum likelihood. The regularization path is computed for the lasso or elasticnet penalty at a grid of values for the regularization parameter lambda. GLMNET is efficient in determining the most important elements in a matrix. We conducted this analysis in the R package *Glmnet Vignette* (Friedman et al., 2009). For task specific signals, we assigned the number 1–3 to nest maintainers, foragers and displayers, respectively, as the response vectors (y). The relative proportions of CHCs on the legs were used as the input matrix (x). After running command cv.glmnet(x, y, family = "multinomial," type.multinomial = "grouped"), we calculated the coefficient of each component using coef(cvfit, s = "lambda.min") command. This model generated a group of coefficients for each task. The command coef(cvfit, s = "lambda.1se") gives the most regularized model such that error is within one standard error of the minimum, which generates another set of components with a smaller number of effective components (**Supplementary Table 1**). Discriminant analysis of colonies and tasks were conducted using these components. The components with no coefficient are those that have little effect in explaining the task variation. The components with positive coefficients are those that positively correlated to the response, and hence ants with a higher concentration of this component have a high probability of being classified into this task. The components with negative coefficient indicate that ants with a higher content of this component have a lower probability of being classified into this task or colony. The importance of each component is correlated with the absolute value of each coefficient. Similarly, we used colony identity as the response vectors (y), and the relative proportion of CHCs on the antennae as the input matrix (x) to calculate the coefficients for nest-mate recognition signals. The selected components were used to run an additional DA to discriminate colony or task variations using the protocols described above.

RESULTS

Task and Colony Discriminations According to Body-Part Profiles

The chemical profiles of CHCs on the antennae, legs and abdomens differ according to the task of the worker (**Figures 1A–C**), but the legs provide qualitatively greater task discrimination accuracy (81.4% correct classification) than either the antennae (71.7% correct classification) or abdomens (69.5% correct classification). The chemical profiles of the three body parts also show clear colony discrimination (antennae: 78.3% correct classification, legs: 84.8% correct classification, abdomens: 72.3% correct classification; **Supplementary Figures S1A–C**).

Task Allocation and Body Size

The head width of workers, used as a measure of body size, differs significantly according to worker task [$F_{(2,250)} = 12.29$, $p < 0.0001$; REML variance component estimate of colony identity: Wald $p = 0.1025$, 27.2% of the total variance; **Figure 1D**]. However, body size seems unlikely to be used as a cue for task discrimination because the effect size is very small (displayers head width 1.994 ± 0.012 mm, foragers 1.955 ± 0.010 mm, nest maintainers 1.939 ± 0.011 mm). While task discrimination can be linked with body size (for example, physical castes) it is also associated with chemical signals (Kaib et al., 2000) and sensory structures associated with chemical communication (Wittwer and Elgar, 2018).

Workers Can Discriminate Tasks

Our behavior assays indicate that workers of *I. purpureus* vary their response to non-nestmates according to the task of their opponent, rather than their own task. The frequency of antennation behavior varied according to the task of the stimulus worker [$F_{(2,107)} = 35.43$, $p < 0.0001$; REML Variance component estimate of colony identity: Wald $p = 0.1433$, 39.5% of the total variance], with the highest antennation frequency directed toward displayers and foragers than to nest maintainers (**Figure 2A**). The duration of display behavior of the focal worker varied according to the task of the stimulus worker [$F_{(2,100)} = 14.393$, $p < 0.0001$; REML Variance component estimate of colony identity: Wald $p = 0.144$, 40.6% of the total variance]. In contrast, the behavior of the stimulus workers did not vary with the task they were undertaking [$F_{(2,100)} = 1.01$, $p = 0.37$; REML Variance component estimate of colony identity: Wald $p = 0.155$, 33.2% of the total variance; **Figure 2B**]. Thus, workers of *I. purpureus* react differently to different tasks of non-nestmate workers, regardless of their own tasks.

Task Discrimination Signals Are Located on the Legs

The different responses of focal workers to stimulus workers may reflect differences in the behavior of the stimulus workers, rather than a capacity to perceive different CHC profiles and respond appropriately. We compared the duration of display behaviors by focal ants toward non-nestmate stimulus workers that had been engaged in nest maintenance, foraging or displaying, and

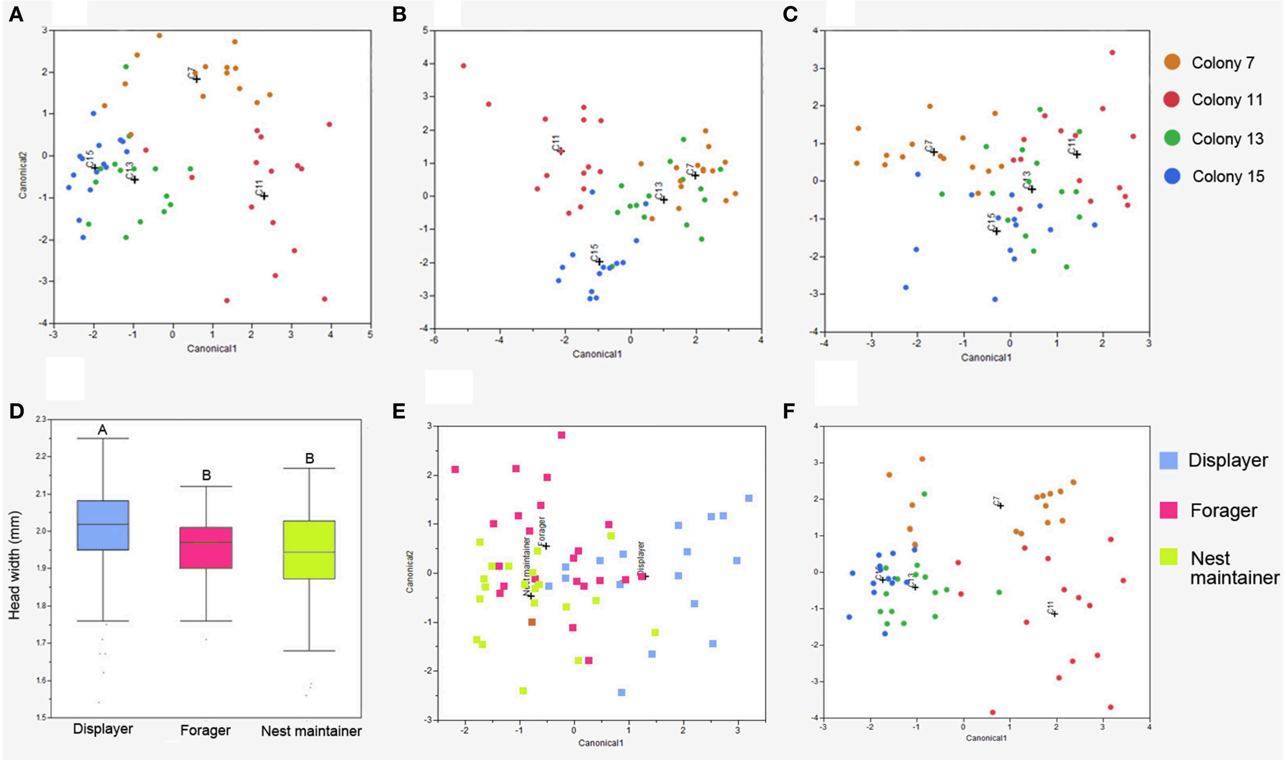

FIGURE 1 | Discriminant analysis of the CHCs on different body parts and body size variation between tasks. Task discriminant analysis plots of **(A)** antennae, correctly classifies 71.7% individuals, **(B)** legs, correctly classifies 81.4% individuals, and **(C)** abdomens, correctly classifies 69.5% individuals. **(D)** Displaying workers are slightly larger than workers engaged in other tasks: box covers 25–75% quartiles and whiskers complete the range of values; different letters indicate significantly (p < 0.05) different levels. **(E)** Task and **(F)** colony discriminant analysis plots using the components selected by GLMNET from legs (correctly classifies 78.0% individuals) and antennae (correctly classifies 86.4% individuals). + marks the centroid of each group.

whose CHCs had been removed on the legs of either one (three legs) or both (six legs) sides of their body. The variation in the duration of display behavior was explained by a significant task × experimental treatment interaction term [$F_{(2,226)} = 12.420$, $p < 0.0001$; REML Variance component estimate of colony identity: Wald $p = 0.1882$, 22.9% of the total variance; **Figure 2C**]. Specifically, the mean duration of displays with forager and displayer stimulus workers was significantly lower when six than three legs had been washed. However, the signal removal treatment had no effect on the mean duration of displays with nest maintainer workers. These results indicate that the variation in the response of the focal ant arises through differences in the chemical signal rather than the behavior of the stimulus workers.

Identification of Active Compounds

We used GLMNET to identify which compounds contributed the most to distinguishing between the CHC profiles on the legs of workers engaged in different tasks. This analysis identified 21 compounds from the CHC profile of the leg samples, including 16 branched, and five linear types that are inferred to be important in explaining task variation. The same analysis identified 17 compounds from the antennal profile that possibly serve as colony identity signals, including 12 branched alkanes, and five linear types (**Table 1**). The importance of each component is correlated with the absolute value of each coefficient, the components with a positive coefficient indicate that ants with a higher content of this component have a high probability of being classified into this task or colony, and the components with a negative coefficient indicate that ants with a higher content of this component have a lower probability of being classified into this task or colony. Interestingly, task and colony recognition signals have very different active components, with only nine overlapping in both signals. On the other hand, the overlap in the compounds identified for task and colony identity signals indicate that ants from different colonies may use the same set of compounds to convey specific signals.

Confining the DA analysis to only the components identified by GLMNET yielded broadly similar discrimination success: 86.4% of the individuals were classified into the right tasks using selected leg CHCs (**Figure 1E**), and 78.0% of the individuals were classified into the right colony using selected antennal CHCs (**Figure 1F**; **Supplementary Material**).

DISCUSSION

Workers of *I. purpureus* can perceive task recognition signals of non-nestmate workers, and respond accordingly. Not only

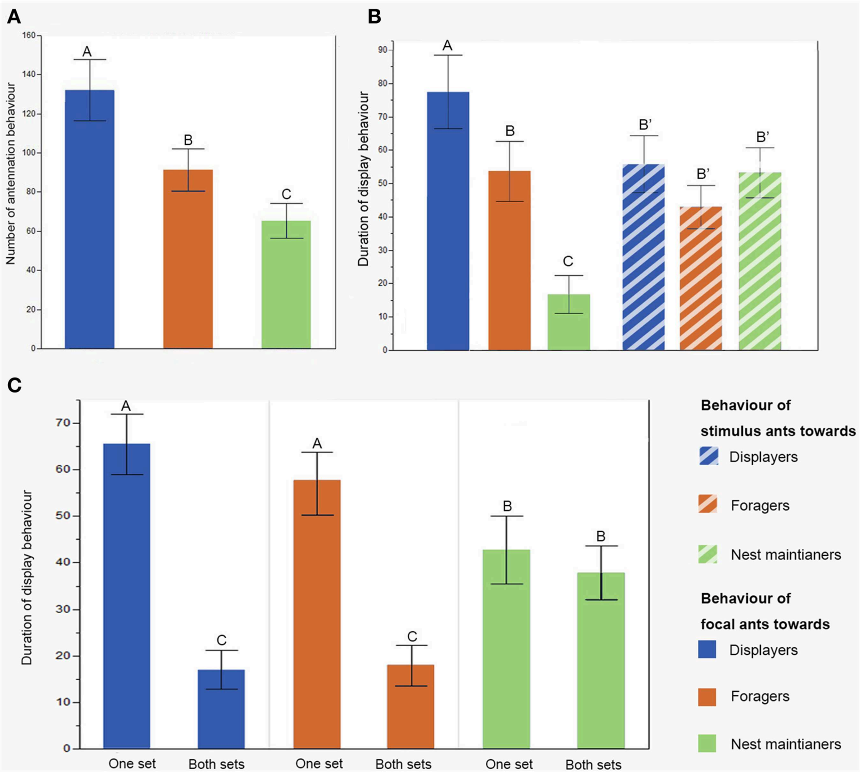

FIGURE 2 | Antennation and aggression behavior of the focal ants toward ants of the three task groups. **(A)** The variation in antennation frequency of the focal ants toward the three task groups; **(B)** the duration of display behavior of the focal ants toward the three stimulus task groups, and the stimulus ants toward the focal ants. **(C)** Focal ants cannot discriminate tasks when the CHCs on the legs were removed in displayers and foragers, as the level of aggression was significantly reduced (tasks: $F_{2,242} = 0.416$, $p = 0.6605$; leg wash: $F_{1,242} = 53.56$, $p < 0.0001$; task × leg wash: $F_{2,242} = 9.506$, $p = 0.0001$; error bars indicate SEM, different letters signify significantly different levels).

are the CHC profiles of the antennae, legs, and abdomens different (Wang et al., 2016), but they also differ according to the workers' task. This ability is lost when the CHCs on the legs are experimentally removed, indicating that these CHCs are vital for task recognition. The chemical components contributing the most to task and colony recognition signals are significantly different. Further analysis using only these particular chemical components shows improved statistical success in task discrimination and similar colony discrimination. This suggests that different signals can be conveyed by a limited number of particular components of the CHCs, greatly narrowing the search to identify the cuticle-based chemicals that represent task and other recognition signals.

The ability to discriminate between non-nestmate workers according to their task allows workers to respond to different levels of threat faced by the colony. Nest maintenance workers of *I. purpureus* pose little threat to other colonies because this task is undertaken close to the nest entrances. In contrast, foraging workers may threaten food resources, and thus pose a higher threat, while displayers, typically engaged in colony defense or offense, pose the greatest threat. The ability of workers to perceive displaying, non-nestmate workers, and respond appropriately, may be particularly important if display behavior provides a signal of individual or colony-level competitive ability (van Wilgenburg et al., 2005).

Our experiments indicate that the CHCs on the legs are crucial for task recognition in *I. purpureus*, expanding the evidence that cuticular-based signaling in social insects is likely to be location specific. Consistent with other task-specific, identity signals (e.g., the queen mandibular pheromone, Dietemann et al., 2003), task recognition signals do not appear to be colony specific. However, while the queens' task remains largely unchanged during her life, workers can change their task according to either long-term colony needs, or short-term emergencies (Gordon, 1989). It is unclear whether the task recognition signals change before or after a change in task, which raises the intriguing question of how the workers change the task recognition signals on their legs. Age induced hormone or gene expression changes are associated with activities of exocrine glands in social insects involved in different tasks (Lengyel et al., 2007; Ueno et al., 2015), and these changes could influence the CHCs that serve as task recognition signals (Lengyel et al., 2007). The environment may also influence task recognition signals. For example, the CHC profile on the legs of *Lasius niger* is similar to that on the surface of the nest (Lenoir et al., 2009): perhaps task recognition signals are at least partly derived from the surface that each insect commonly encounters whilst completing its task.

TABLE 1 | GLMNET coefficient of compounds on the antennae and legs of *Iridomyrmex purpureus* that explain task and nestmate variation.

Compound	Antenna					Leg			
	% Mean (±SE)	Coefficient				% Mean (±SE)	Coefficient		
		Colony 1	Colony 2	Colony 3	Colony 4		Nest maintainer	Forager	Displayer
n-nonadecane	7.10 ± 0.86	.	.	.	.	3.63 ± 0.97	18.79	−1.62	−17.17
n-henicosane	1.28 ± 0.41	−13.80	33.10	−6.28	−13.02	2.11 ± 0.91	•	•	•
n-docosane	—					2.91 ± 1.11	−26.83	38.75	−11.92
Hydrocarbon with C_{22} backbone	—					0.65 ± 0.17	•	•	•
9-docosane	1.52 ± 0.36	46.26	−12.08	−13.95	−20.22	0.75 ± 0.27	−24.37	61.68	−37.31
n-tricosane	2.89 ± 1.01	−41.40	35.10	15.00	−8.71	7.30 ± 3.08	•	•	•
9- or 13-tricosane	9.98 ± 2.4	−0.39	2.02	−1.79	0.17	0.51 ± 0.54	•	•	•
Hydrocarbon with C_{23} backbone	3.76 ± 1.85	•	•	•	•	5.79 ± 1.43	•	•	•
10- or 14-tricosane	15.61 ± 2.49	•	•	•	•	7.62 ± 1.61	•	•	•
n-tetracosane	—					1.33 ± 0.47	•	•	•
1,21-docosadiene	—					0.80 ± 0.27	•	•	•
Hydrocarbon with C_{24} backbone	1.32 ± 0.43	−148.42	112.69	147.54	−111.80	2.48 ± 0.78	−2.12	−0.69	2.81
n-pentacosane	10.32 ± 1.41	•	•	•	•	4.86 ± 1.08	•	•	•
11- or 13-pentacosane	—					0.69 ± 0.16	7.31	−1.68	−5.63
11, 15- or 10, 14-dimethyl pentacosane	—					0.85 ± 0.38	•	•	•
n-hexacosane	—					0.42 ± 0.19	•	•	•
n-heptacosane	—					2.40 ± 1.01	•	•	•
11- or 13-heptacosane	—					0.48 ± 0.69	•	•	•
7, 11- or 7, 15-dimethyl heptacosane	9.19 ± 3.57	15.36	−12.12	6.50	−9.73	2.92 ± 0.96	2.20	−0.68	−1.52
n-octacosane	—					0.78 ± 0.28	•	•	•
12, 14- or 16, 18-dimethyl octacosane	—					1.30 ± 0.73	−1.8	−0.04	1.85
Hydrocarbon with C_{28} backbone	2.74 ± 1.55	•	•	•	•	5.04 ± 2.95	•	•	•
n-nonacosane	0.44 ± 0.19	4.10	19.56	−126.17	102.51	1.33 ± 0.38	−6.82	9.10	−2.28
11- or 13- or 15-nonacosane	—					1.17 ± 0.36	•	•	•
11, 15- or 13, 17-dimethyl nonacosane	1.08 ± 0.27	−1.82	78.75	−128.88	48.93	0.72 ± 0.13	−1.30	−15.64	16.94
n-triacontane	0.18 ± 0.17	−0.01	−22.26	28.20	−5.94	0.48 ± 0.19	•	•	•
11- or 13- or 15-triacontane	0.53 ± 0.33	•	•	•	•	1.73 ± 0.33	•	•	•
11, 15- or 13, 17-dimethyl triacontane	2.00 ± 1.21	3.25	0.54	2.96	−6.76	2.73 ± 1.84	•	•	•
n-hentriacontane	—					0.57 ± 0.4	•	•	•
Hydrocarbon with C_{31} backbone	12.13 ± 8.1	−14.67	−14.20	10.02	18.86	10.88 ± 2.66	10.36	6.23	−16.59

(Continued)

TABLE 1 | Continued

Compound	Antenna					Leg			
	% Mean (±SE)	Coefficient				% Mean (±SE)	Coefficient		
		Colony 1	Colony 2	Colony 3	Colony 4		Nest maintainer	Forager	Displayer
11- or 13- or 15-hentriacontane	1.16 ± 0.61	12.28	96.56	−37.23	−71.60	5.47 ± 1.49	•	•	•
11, 15- or 13, 17-dimethyl hentriacontane	0.34 ± 0.24	197.79	−150.70	−262.03	214.94	0.49 ± 0.47	•	•	•
n-dotriacontane	0.87 ± 0.19	−0.14	7.59	3.11	−9.29	0.50 ± 0.2	108.34	19.09	−127.43
Hydrocarbon with C_{32} backbone	—					0.99 ± 0.6	−4.00	4.28	−0.29
12- or 13- or 14- or 15- or 16-dotriacontane	—					0.14 ± 0.13	25.99	5.42	−31.41
Hydrocarbon with C_{32} backbone	—					0.08 ± 0.07	•	•	•
Hydrocarbon with C_{32} backbone	—					0.27 ± 0.2	60.69	−4.59	−56.10
Hydrocarbon with C_{33} backbone	1.54 ± 0.9	55.93	−24.99	4.46	−35.39	5.39 ± 1.42	1.99	−5.74	3.75
Hydrocarbon with C_{33} backbone	—					2.08 ± 0.57	−46.35	42.25	4.10
11- or 13- or 15- or 17-tritriacontane	—					0.18 ± 0.12	•	•	•
11, 15- or 13, 17-dimethyl tritriacontane	1.42 ± 0.71	•	•	•	•	0.60 ± 0.23	70.70	−78.79	8.09
Hydrocarbon with C_{34} backbone	—					0.06 ± 0.08	−154.52	202.69	−48.18
11, 13- or 15, 17-dimethyl pentatriacontane	—					0.97 ± 0.26	−35.76	−6.43	42.19
Hydrocarbon with C_{35} backbone	—					1.31 ± 0.55	−0.22	0.12	0.09
Hydrocarbon with C_{35} backbone	10.64 ± 1.78	0.48	−2.18	−13.92	11.28	4.42 ± 2.03	13.15	−7.18	−5.98
Hydrocarbon with C_{35} backbone	1.92 ± 1.07	70.13	−36.80	−41.67	8.35	1.11 ± 0.38	•	•	•
Hydrocarbon with C_{36} backbone	—					0.72 ± 0.37	•	•	•

The coefficients are positively correlated to the response; hence the components with a positive coefficient indicate that ants with a higher content of this component have a high probability of being classified into this task or colony, and the components with a negative coefficient indicate that ants with a lower content of this component have a high probability of being classified into this task or colony. The components with no coefficient number have little effect in explaining the task or colony variation. (—, components undetected; •, components with little or no effect in explaining the variation).

Studies that attempt to identify the chemical compounds involved in communication about task allocation have been dogged by the number of compounds present on the insect cuticle. Our statistical analyses have proved helpful in identifying which components are most closely associated with particular tasks, and thus help narrow the range of potential compounds that may act as task recognition and nest identity signals. The chemical components identified by GLMNET as possible candidates for signaling compounds include mostly branched hydrocarbons, which is consistent with other lines of evidence suggesting that this class of hydrocarbons are likely to have signaling functions (Martin et al., 2008; Martin and Drijfhout, 2009a; van Wilgenburg et al., 2010, 2012). However, this does not indicate that the chemical components highlighted by GLMNET are the actual signals used by the ants: experimental results using synthesized components are still needed to confirm the results. Interestingly, the vast majority of the chemicals with high coefficient values are clearly differentiated between the two signal functions, despite the overlap of the CHC profile across body parts. This suggests that not all components revealed by whole body samples represent signals (Hölldobler et al., 2004; Martin et al., 2008), an arrangement that likely minimizes any signal ambiguity because it allows ants to perceive particular signals from targeted sources, rather than having to distinguish signals from a complex pool of CHCs, as implied in other insects (Chin et al., 2014). Further, these data highlight the importance of recognizing that candidate CHC signals may be location-specific, and are thus obscured by conventional methods of analyzing the CHCs extracted from the entire insect cuticle.

ETHICS STATEMENT

No animal ethics approval was required for this study, and permission was granted to conduct behavioral assays at Serendip Sanctuary.

AUTHOR CONTRIBUTIONS

QW and ME: conceived and designed the study. QW: performed behavioral assays. QW, JG, and IW: conducted the chemical analysis and interpretation. LC, QW, and ME: conducted the statistical analyses. QW and ME: drafted the manuscript. QW, JG, IW, LC, and ME: edited and revised 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.2019.00227/full#supplementary-material

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Mechanisms of Social Influence: A Meta-Analysis of the Effects of Social Information on Female Mate Choice Decisions

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Social learning about mate choices is taxonomically widespread, and is a potentially important mechanism of social evolution that may affect the strength of sexual selection in a population. We used a meta-analytic approach to estimate the effect of mate-choice copying on reproductive decisions. We evaluated effect sizes across 103 experiments from 40 studies that experimentally measured female mate-choice copying in non-human animals representing Arachnida, Insecta, Malacostraca, Aves, and Actinopterygii. Our goals were to quantify the magnitude of the effect of this form of social influence, and the extent to which it is modified by observer experience, model age relative to the observer, attractiveness of prospective mates, and testing conditions (laboratory vs. free-living). Across all studies, females that observed others choosing a male were on average 2.71 times more likely to mate with that male, or with a phenotypically similar individual, compared to females with no social information (odds ratio 95% credible interval: 1.60–4.80). After corrected for publication bias, this effect remained significant (corrected odds ratio: 1.92, 95% credible interval 1.13–3.40). We found little evidence for phylogenetic effects in the occurrence of mate-choice copying. Indeed, studies herein present evidence for mate-choice copying in a broad cross-section of species, but also report exceptions in sister taxa. Social information from observed mate choices of others had a considerably stronger effect on mate choice in free-living subjects than in captive individuals. Inexperienced (virgin) females were more likely to copy mate choices than were experienced females, but the relative age of the model was unrelated to whether copying occurred. Finally, females were more likely to copy the mate choices of others when social information counteracted the observing female's personal or genetic mating preference. We note the need for increased taxonomic representation in tests of mate-choice copying, given the robust demonstration of effects in taxa studied to date. Such broader information will provide additional insight to the drivers of the differences identified here in tendency to copy mate choices of others.

Keywords: cultural inheritance, *Drosophila*, mate-choice behavior, mate-choice copying, sexual selection, social learning, meta-analytic, *Poecilia*

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INTRODUCTION

One of the major benefits of living in social groups is access to information. Individuals can employ social information to recognize appropriate habitats, food items, and mates, thereby navigating some of the most important decisions facing an animal (Galef and Giraldeau, 2001; Danchin et al., 2004). When individuals use information gleaned from observing conspecifics (Hoppitt and Laland, 2008), they can reduce decision time, time to discover scarce resources, and costs of assessing competing choices (Valone and Templeton, 2002; Lee et al., 2016). In the context of mate choice, the extent to which animals change their mate choices in response to the behavior of others can influence the strength and dynamics of sexual selection in a population (Wade and Pruett-Jones, 1990; Verzijden et al., 2012). As such, socially mediated mate choices can modify the course of evolutionary change.

Female mate-choice copying is a type of social learning that occurs when a female's likelihood of mating with a male is influenced by the apparent choices of other females. Early consideration of this phenomenon suggested copying may be a factor in the high reproductive skew among lekking male sage grouse (*Centrocercus urophasianus*; Wiley, 1973) and white-bearded manakins (*Manacus manacus*; Lill, 1974), but the occurrence of mate-choice copying was first demonstrated experimentally in a captive population of Trinidadian guppies (*Poecilia reticulata*; Dugatkin, 1992). Regular reviews in the subsequent decades indicate the sustained interest in mate-choice copying and its implications for sexual selection (Gibson and Höglund, 1992; Nordell and Valone, 1998; Vakirtzis, 2011; Witte et al., 2015; Varela et al., 2018). Mate-choice copying occurs in a wide array of organisms, from humans (Waynforth, 2007; Gouda-Vossos et al., 2018; *Homo sapiens*) to *Schizocosa* wolf spiders (Fowler-Finn et al., 2015), including in animals that lack complex cognitive abilities or social systems (Danchin et al., 2018). Given the apparent prevalence and importance of mate-choice copying, we aimed to assess the magnitude of its effects as well as the factors that influence this phenomenon.

Theoretical treatments predict that mate-choice copying should be favored in situations where information is limited, or when choice is costly (Gibson and Höglund, 1992; Pruett-Jones, 1992). The perspective that mate-choice copying serves as a mechanism to reduce observer uncertainty has received the strongest support to date (Vakirtzis, 2011). In taxonomically diverse animals, and in a variety of situations other than mate choice, social information has a greater influence when observers are young or otherwise inexperienced, for example when they encounter new environments or when a preferred choice is unavailable (Galef and Giraldeau, 2001; Valone and Templeton, 2002; but see Loukola et al., 2012). In mate-choice copying, this suggests that young or sexually inexperienced females should be more likely to copy the mate choices of others, as has been reported in guppies and wolf spiders (Dugatkin and Godin, 1993; Fowler-Finn et al., 2015).

Empirical studies to date have also demonstrated that all social information is not equal. Observers of many species seem to pay more attention to information from certain individuals. For example, captive chimpanzees (*Pan troglodytes*) were more likely to learn a novel foraging behavior form socially dominant and knowledgeable individuals (Kendal et al., 2015), and humans are more likely to copy mate choices of more desirable demonstrators (Gouda-Vossos et al., 2018). In addition to being influenced by characteristics of observers, mate-choice copying decisions may vary based on characteristics of the model females.

Relative quality of choices is also a factor that can influence the degree to which individuals use social information in decision making. For example, in ocellated wrasse (*Symphodus ocellatus*), mate-choice copying is more likely to occur when model females are observed with unattractive males rather than with attractive ones (Alonzo, 2008). Across taxa, approaches to quantifying mate-choice copying have differed in whether an observer's innate preference was incorporated into measures of the influence of social information. Studies employing random enhancement increase attractiveness of a particular male using signals of other females' preferences. Enhancement studies usually choose the male at random for the addition of the conspecific cue and assume that without that information females would be equally likely to choose between the two males (Dugatkin, 1992; Germain et al., 2016). In studies that incorporate "reversals," experimenters first identify a female's preferred mate, either by directly quantifying preference for one of two competing males (Dugatkin and Godin, 1992), or by using prior evidence of genetically determined mating preferences (Dugatkin, 1996, 1998). They then quantify whether providing social cues in favor of the non-preferred male could change expressed mate choices. Because the personal or genetic preference of a female is known, reversal studies may produce less ambiguous results about the strength of mate-choice copying.

Given the difficulty of monitoring female movements and information in the wild, mate-choice copying has been studied almost exclusively in laboratory situations. However, work in wild populations also indicates that copying occurs outside of the lab. Some studies have manipulated behavior in the wild in ways comparable to lab experiments, by adding model females or other signals of mate preference to free-living but stationary males, and quantifying change in the behavior of other females that results (Höglund et al., 1995; Stiver and Alonzo, 2010). In other cases, mobile males have been restrained in tanks, with model females placed in close proximity, as is commonly done in laboratory trials, but unrestrained females were allowed to approach the restrained males under otherwise natural conditions (Witte and Ryan, 2002; Godin and Hair, 2009). Given that mate-choice copying is an ecologically relevant phenomenon expected to influence the behavior and ultimate fitness of animals, we aimed to quantify if our understanding of mate-choice copying in the laboratory is comparable to how it functions in nature.

While tests of mate-choice copying commonly quantify influence on choice of specific males, recent work demonstrates that females learn general characteristics of target males and copy choices of phenotypes, rather than of specific individuals (Witte and Noltemeier, 2002; Godin et al., 2005; Danchin et al., 2018). This distinction is important for understanding how broadly and rapidly mate-choice copying could affect patterns of sexual selection in real populations. When model females were associated with males of a specific phenotype of a visible morphology, observers demonstrated a generalized preference for the same phenotype rather than a preference for the specific individual male (Kniel et al., 2015; Dagaeff et al., 2016; Nöbel et al., 2018). For example, when female *Drosophila* observed others mating with males covered in either pink or green powder, they formed a preference for these novel, artificially generated color phenotypes when they encountered them in different males, even when new males differed in other obvious phenotypes (Danchin et al., 2018). What's more, this phenotype-specific preference persisted over time, intensified after a 24-h consolidation period, and was lost if females were treated with a protein synthesis inhibitor that prevented long-term memory formation (Danchin et al., 2018).

Given the potential importance of mate-choice copying in sexual selection, our goals in this study were to determine the magnitude of the effect of mate-choice copying across studies, and to test whether the strength of copying effects is modified by observer characteristics, demonstrator characteristics, and effects of differences in attractiveness among competing choices. We also tested whether the effect of mate-choice copying differed between captive and free-living systems. We used a meta-analytic approach that allowed us to control for non-independent data due to phylogenetic relationships, taxonomy, and multiple experiments conducted during the same study. We also assessed the level of heterogeneity in mate-choice copying effects. Finally, we tested for the occurrence and impact of publication bias in the study of mate-choice copying.

A recent review and meta-analysis of effects in studies of human mate-choice copying found that effects were strongest in females, and that both males and females rated prospective mates as more desirable when they were in the presence of more desirable models (Gouda-Vossos et al., 2018). Most human studies indirectly assess mating preferences, for example by having subjects rate the relative attractiveness of a person on a numeric scale, which is not easily combined with assessment metrics in non-human animals. Here we focused on female mate-choice copying in non-human animals. While males of some species may copy the mate-choice of other males (Schlupp and Ryan, 1997; Witte and Ryan, 2002; Widemo, 2005), we focus here on females both because there have been relatively few studies on this phenomenon in males, and because the sexes may diverge in strategies employed in choosing mates. For example, males of some species avoid recently mated females, with whom chances of fertilization may be lower (White and Galef, 1999). Additionally, audience effects are often apparent, as when males reverse mating preferences in the presence of other males, arguably decoying others away from their preferred mates and reducing pre- or post-copulatory competition for fertilizations (Bierbach et al., 2011; Auld and Godin, 2015).

Our study is, to the best of our knowledge, the first meta-analysis of female mate-choice copying in non-human animals. This work aims to more clearly define the extent to which individuals pay attention to social information during mate choice.

METHODS

We conducted a meta-analysis of empirical studies quantifying mate-choice copying in non-human animals. We identified relevant studies using a topic search in Web of Science (ISI Thomas Reuters) specifying the search criteria as: TS = [("mate choice" AND "copy*") OR ("mate-choice" AND "copy*") OR "mate-copy*" OR "mate copy*" OR "female* copy*"], targeting peer-reviewed research published between 1900 and May 2019. This returned a total of 356 publications. Of these, we excluded 102 human studies. We then filtered out 113 articles that were clearly not about mate-choice copying and 51 articles that lacked empirical data (e.g., reviews, theoretical models, or opinion papers). We also excluded 14 studies of the audience effect on male mate-choice behavior, in which males reverse apparent mating preferences in the presence of other males (Bierbach et al., 2011; Auld and Godin, 2015). We excluded nine studies that did not experimentally assess mate-choice copying directly or at all. We further excluded 24 studies that assessed mate-choice copying but did not determine individual choices. Finally, we excluded three studies quantifying male mate-choice copying because these studies identified audience effects (described above) on male behavior that are logically separate from selective pressures on female behavior. We were left with 40 empirical studies reporting 103 separate experiments that tested directly for female mate-choice copying (**Figure 1**).

Extraction of Effect Sizes

Studies considered for this meta-analysis quantified mate-choice copying by allowing an observer female to choose between two males, one of which was previously associated (e.g., via spatial proximity, courtship behavior, or copulation) with another female (i.e., the model). The choices of observers with the opportunity to mate-choice copy were compared to one of two types of controls: (1) observer females that did not witness the model female's choice, and thus did not have the opportunity to mate-choice copy ($n = 43$) or (2) an assumed 50/50 odds that an observer would pick either of the two males in the absence of a mate-choice copying opportunity ($n = 60$). In both cases, we extracted effect sizes as odds ratios (OR) with the following formula:

$$OR = \frac{AD}{BC} \tag{1}$$

A is the number of female observers exposed to a model's choice that chose the same male or male phenotype. Similarly, B is the number of observers exposed to a model's choice that did not copy it. C is the number of control females (i.e., females that did not witness the model's choice) that made the same choice as the model. D is the number of controls that did not choose the same as the model. We used the natural log of the OR [$ln(OR)$] to normalize the distribution of the data. We back transformed all model estimates and credible intervals to OR to more intuitively report the results. We calculated variance [$V_{ln(OR)}$] and sampling error [$SE_{ln(OR)}$] of the $ln\,(OR)$ of each

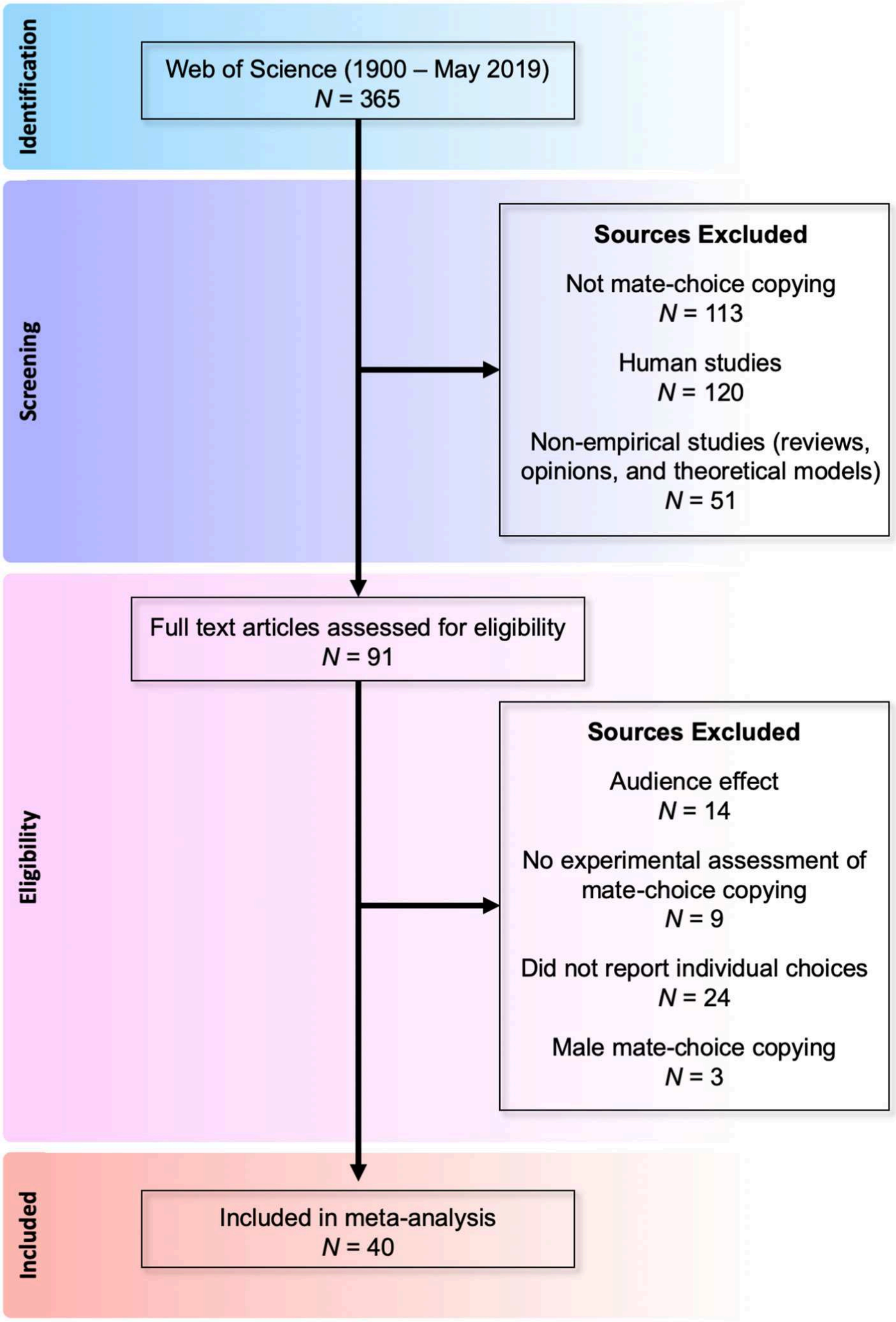

FIGURE 1 | Flow diagram of selection process for female mate-choice studies.

experiment with the follow equations:

$$V_{ln(OR)} = \frac{1}{A} + \frac{1}{B} + \frac{1}{C} + \frac{1}{D} \tag{2}$$

$$SE_{ln(OR)} = \sqrt{V_{ln(OR)}} \tag{3}$$

The parameters $A - D$ are the same as described above. Note that as variance for $ln\ (OR)$ is determined solely on the sample size of each group, experiments with small samples sizes will have large sample errors. Some studies did not use a control group *per se*, but rather assumed 50:50 odds of choosing either male. In these cases, we calculated OR, $V_{ln(OR)}$, and $SE_{ln(OR)}$ using hypothetical values for C (C_h) and D (D_h) based on the same number of individuals exposed to the model choice and the 50% probability assumption (Equation 4).

$$C_h = D_h = \frac{A + B}{2} \tag{4}$$

Coding Moderators of Mate-Choice Copying Behavior

We identified four key factors that we hypothesized modify the strength of mate-choice copying effects and that we were able to assess for all studies in our sample: observer experience, model female age, male attractiveness, and testing conditions (i.e., free-living vs. captive).

Observer experience was categorized as virgin (females with no prior sexual experience) or non-virgin. In this latter condition, we assumed that females housed in mixed-sex groups were likely non-virgins unless the author(s) explicitly stated that mixed groups were held at environmental parameters to suppress breeding condition development. It is possible that mixed-sex groups included both virgin and non-virgin individuals.

Model female age was relative to the observer's age and categorized as "older" or "same or younger." Size was reasonably used as a proxy for age in studies of fish, such as guppies, that exhibit indeterminate growth. Only two in our set of 103 retained experiments used a model that was younger relative to the female observer (Dugatkin and Godin, 1993; Vukomanovic and Rodd, 2007).

Male attractiveness was estimated by whether the experiment provided social information favoring the relatively less attractive of two males. Many experimental protocols used a reversal paradigm, in which a female was paired with a male previously rejected by the observer. We considered these rejected males to be less attractive in the eyes of the observer but nonetheless "chosen" by the model during copying trials. A subset of studies used previously identified mating preferences of females from the study population to define unattractive male characteristics (e.g., dull male guppies from a population in which females prefer bright orange males, Dugatkin, 1998). In experiments in which social information did not favor less attractive males, model females were either paired with a randomly chosen male (usually from a phenotypically matched pair of males) or, more rarely, allowed to choose independently. In these experiments, males paired with a model were considered no more or less attractive, on average.

Finally, we parameterized testing conditions as whether experiments were conducted in captivity or in a natural setting with free-living individuals. Our captive category included both lab-reared individuals, and those that were wild-caught and tested in the laboratory. Extracted data used in this meta-analysis are provided in the Supplementary Materials (**Table S1**).

Meta-Analysis

We conducted a meta-analysis of results from mate-choice copying studies using univariate-response Bayesian generalized linear models with Markov chain Monte Carlo (Hadfield, 2010; Hadfield and Nakagawa, 2010) in the programing language R, version 3.6.0 (R Core Team, 2019) and *MCMCglmm* package (Hadfield, 2010). Under this framework, we compared a random-effects model to a mixed-effects model (i.e., meta-regression; Hedges and Vevea, 1998). The random-effects model included a matrix of phylogenetic distances, and random effects of study and species (see below for full justification). The mixed-effects model included those random effects and also our four hypothesized explanatory factors described above (observer experience, demonstrator age, male attractiveness, and testing conditions). These models did not assume a single true effect size for mate-choice copying. Rather, they assumed that each individual study had a true effect size and thus estimated the mean of the distribution of effect sizes. The mixed effects models also weighted individual effect sizes by the inverse of $SE_{ln(OR)}$, so that experiments with larger sample sizes had a stronger influence on the parameter estimates. First, we quantified heterogeneity in our random-effects model and then the mixed-effects model (Higgins and Thompson, 2002; Higgins et al., 2003) to determine how well-moderators (i.e., explanatory factors) accounted for effect size variation among experiments. Second, we compared the fit of these two models using Deviance Information Criterion (DIC; Spiegelhalter et al., 2014). Smaller DIC values indicate a better overall fit of the model to the data. Third, we determined the overall mean effect size of mate-choice copying and tested the significance of explanatory moderators. Lastly, we assessed possible publication bias (Sutton, 2009; Nakagawa and Santos, 2012).

Phylogenetic and Taxonomic Meta-Analytic Model

Meta-regressions are particularly useful in ecology and evolution, as they can accommodate non-independent data due to phylogenetic relationships among taxa and when individual studies each produce multiple effect sizes (Nakagawa and Santos, 2012). Controlling for phylogeny in comparative analyses is essential (Freckleton et al., 2002; Blomberg et al., 2003), and increasingly advocated in biological meta-analyses (Adams, 2008; Lajeunesse, 2009; Hadfield and Nakagawa, 2010; Nakagawa and Santos, 2012). However, our dataset included 17 species that spanned five classes within Chordata and Arthropoda. As such, a tree that was fully resolved to the species level was not obtainable. Therefore, we combined phylogenetic and multilevel taxonomic approaches as described by Hadfield and Nakagawa (2010). The phylogenetic portion of our mixed meta-analytic model uses the "animal model" framework (Henderson, 1976) to construct a phylogenetic relatedness matrix and to account for evolutionary divergence times between any two taxa (Lynch, 1991; Pagel, 1999). The relatedness matrix was derived from an ultrametric tree obtained from TimeTree.org (Kumar et al., 2017) and fully resolved to the family level of our 17 unique species (13 families). The phylogenetic control was combined with a taxonomic analysis by including species identity as a random effect. We also included study as a random effect, as many publications had multiple experiments with associated effect sizes.

Posterior Means and Moderators of Mate-Choice Copying

We determined the overall effect size of female mate-choice copying using a univariate-response Bayesian generalized linear mixed-effects model with Markov chain Monte Carlo (BMM; Hadfield, 2010; Hadfield and Nakagawa, 2010). We quantified the posterior mean of the overall *OR* and 95% credible interval of mate-choice copying as well as the 95% prediction interval (Riley et al., 2011). Credible intervals that did not cross an *OR* of 1 were considered statistically significant (*OR* of 1 equals a 50% probability). We also include our four moderators of mate-choice copying as fixed effects and calculated the change in posterior mean *OR* (ΔOR) between their levels (e.g., virgin vs. non-virgin observers) as well as the 95% credible interval. We considered ΔORs to be statistically significantly different if their credible intervals did not overlap. Further, we calculated the mean *OR* and associated 95% credible interval of each level of the moderators using the moderator estimates weighted by the inverse of $SE_{ln(OR)}$.

Measuring Heterogeneity

Heterogeneity, or the degree of inconsistencies across studies, is a vital component of meta-analytic procedures and is expected to be high in ecology and evolution meta-analyses (Senior et al., 2016). We quantified heterogeneity in OR among different studies and species by calculating the percent of variance explained by each random effect. Traditional measures of heterogeneity in meta-analysis (e.g., Q, I^2) were not originally developed for use in models with multiple random effects (Higgins and Thompson, 2002; Nakagawa and Santos, 2012). Here we use a method developed by Nakagawa and Santos (2012) to measure the heterogeneity within a given random effect as a portion of the total heterogeneity based on the formulation of I^2 (Higgins and Thompson, 2002). The total variance (σ_t^2) of our phylogenetic-taxonomic mixed meta-analytic model can be written as the sum of all the random components (Equation 1)

$$\sigma_t^2 = \sigma_p^2 + \sigma_a^2 + \sigma_s^2 + \sigma_m^2 + \sigma_e^2 \tag{5}$$

Variance parameters include those attributed to phylogenetic effects (σ_p^2), species effects (σ_a^2), study effects (σ_s^2), individual experiment sample error (σ_m^2), and residual error (σ_e^2). We were then able to calculate the proportional amount of heterogeneity at the species level (Equation 6) and the study level (Equation 7).

$$I_a^2 = \frac{\sigma_a^2}{\sigma_t^2} \tag{6}$$

$$I_s^2 = \frac{\sigma_s^2}{\sigma_t^2} \tag{7}$$

We also calculated the phylogenetic signal (H^2) with the following equation (Lynch, 1991):

$$H^2 = \frac{\sigma_p^2}{(\sigma_p^2 + \sigma_a^2 + \sigma_s^2 + \sigma_m^2)} \tag{8}$$

A $H^2 = 0$ indicates that phylogenetic relatedness among taxa does not explain effect size variation, whereas $H^2 = 1$ indicates that the effect sizes of taxa are completely proportional to their phylogenetic relationships.

Publication Bias

Publication bias is an important concern in meta-analyses, as it can influence the validity of results (Rosenthal, 1979; Sutton, 2009). Therefore, we assessed possible publication bias using a modified Egger's regression as described by Nakagawa and Santos (2012), in which measures of precision are compared to corresponding meta-analytic residuals. Unlike the weighted effect sizes, the associated residuals are independent of one another and not influenced by heterogeneity (Nakagawa and Santos, 2012). We corrected for publication bias using the "trim and fill" method (Duval and Tweedie, 2000) using the R package *meta* (Schwarzer, 2007), which removes small studies and imputes new effect sizes of hypothetical experiments to restore symmetry in the funnel plot (Duval, 2005). Asymmetry can also be due to heterogeneity in the data, and thus any correction would erroneously bias the true mean effect size (Peters et al., 2007). Therefore, we used meta-analytic residuals described above to determine the presence of publication bias instead of weighted effect sizes as suggested by Nakagawa and Santos (2012).

RESULTS

We calculated 103 effect sizes from 40 published studies of mate-choice copying that met our selection criteria. The mean posterior *OR* for mate-choice copying was 2.71 (95% credible interval 1.60–4.80; **Figure 2**). In other words, given a dichotomous choice, females were on average 2.71 times more likely to choose a particular male, or male of a similar phenotype, if she observed another female choosing him first, compared to females with no social information. The range of likely values for the overall mean effect of mate-choice copying did not cross the null value ($OR = 1$, indicating 50% probability of such a choice). Therefore, we considered the overall mean effect of mate-choice copying statistically significant. The 95% prediction interval of effect sizes, which more fully describes the range of possible effect sizes within any single study rather than just the mean effect size across all studies, was 0.31 to 22.75 (**Figure 2**; see discussion for further interpretation).

Phylogenetic Effects

Our analysis included experiments on 17 species from 13 different families, representing three classes of arthropods and two classes of vertebrates. Our meta-analytic model accounted for phylogenetic relatedness by incorporating divergence times at the family level. We found little evidence for a phylogenetic signal in mate-choice copying ($H^2 = 0.06$, **Table 1**).

Study and Species Heterogeneity

Our initial random meta-analytic model indicated low heterogeneity among individual studies ($I_s^2 = 0.01$) but substantial heterogeneity among species ($I_a^2 = 0.75$, **Table 1**) producing a high level of overall heterogeneity in our data set ($I_a^2 + I_s^2 = 0.76$, **Table 1**; Higgins et al., 2003). In contrast, the inclusion of explanatory factors (i.e., in the mixed-effect meta-analytic model), explained the vast majority of heterogeneity previously attributed to the level of species ($I_a^2 = 0.07$, **Table 1**). Further, DIC values indicated that the mixed-effects model was a slightly better fit given our dataset compared to the random effects model (**Table 1**). However, models that differ in DIC value

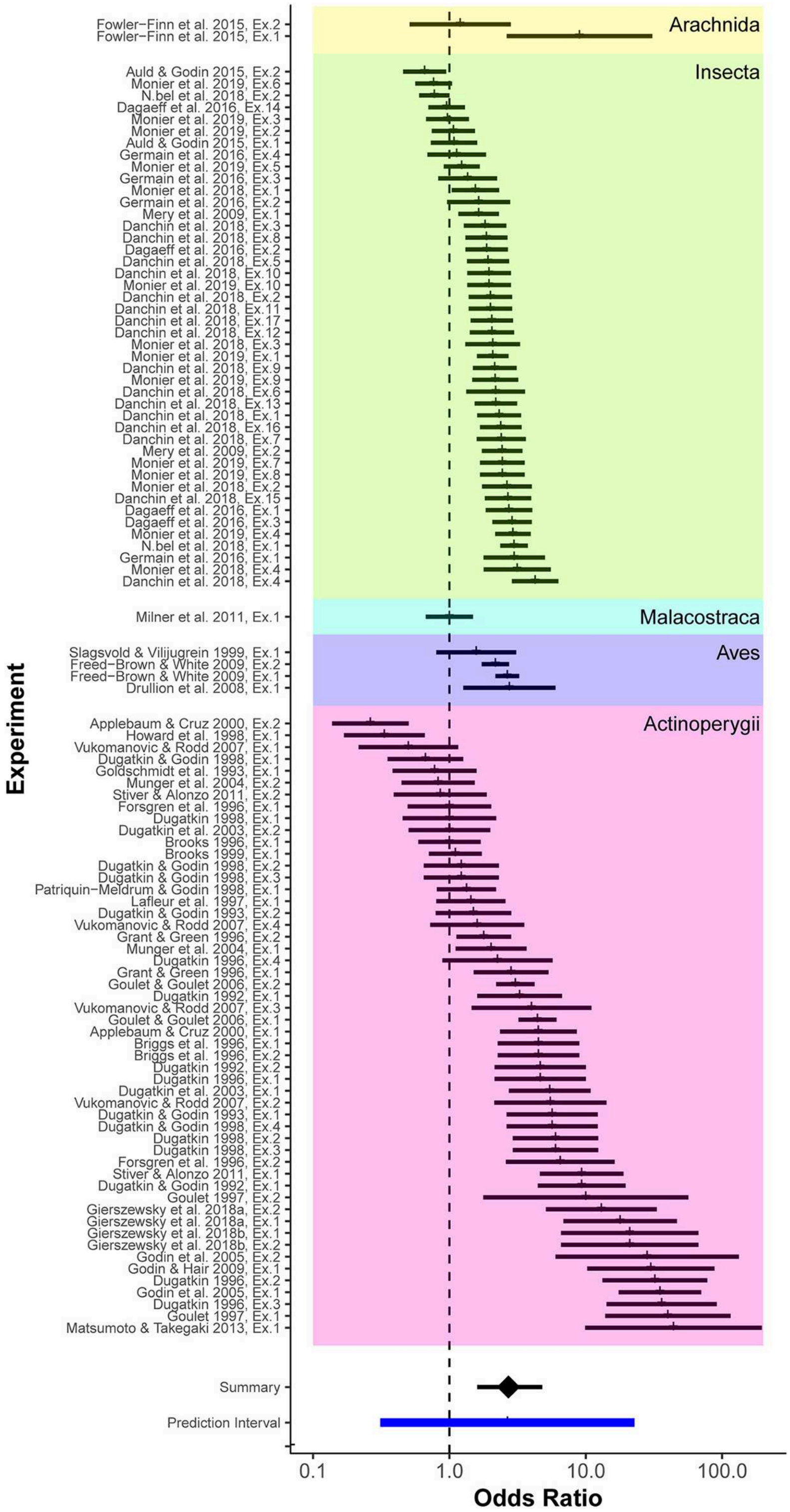

FIGURE 2 | Forest plot of effect sizes (odds ratios) from all considered studies, grouped taxonomically. Colored groupings represent taxonomic divisions by class. From top to bottom these are: Arachnida, Insecta, Malacostraca, Aves, and Actinoperygii. Effect sizes for individual experiments are indicated as vertical tick marks along with associated sampling errors (horizontal bar). The overall posterior mean effect size, derived from the mixed-effects model that accounted for phylogenetic distance, species, and study (**Table 1**), was 2.71, indicating that an average female receiving positive social information about a prospective mate was 2.71 times more likely to mate with that male compared to females with no social information.

TABLE 1 | Heterogeneity explained by random effects in competing meta-analytic models.

Meta-analytic model	Fixed effects	DIC	Percent heterogeneity			Phylogenetic signal (H^2)
			Study ($I_s^2 * 100$)	Species ($I_a^2 * 100$)	Total	
Random	—	278.24	0.7	75.0	75.7	0.05
Mixed	Model female age, male attractiveness, observer experience, testing conditions	276.65	0.8	7.4	8.2	0.06

A model containing only random effects was compared to one including four fixed effects in addition to the same random effects of study, species, and phylogenetic relatedness. See ***Table 2*** *for further information and parameter values for listed fixed effects. Study identity was defined at the level of individual publications and was included to account for non-independence when multiple effect sizes were taken from the same paper. Species was included as a random effect to control for repeated measures at this taxonomic level. Phylogenetic signal was parameterized as a phylogenetic relatedness matrix (see methods) to account for non-independence due to taxonomic divergence times. Models were compared by deviance information criterion (DIC).*

TABLE 2 | Fixed-effect estimates from the Bayesian hierarchical meta-analysis assessing strength of moderators hypothesized to affect the occurrence of mate-choice copying.

Parameter	Estimate	Lower 95% CI	Upper 95% CI	pMCMC
Intercept	1.13	0.65	1.02	0.65
Observer experience (virgin)	**1.53**	**0.16**	**2.94**	**0.03**
Model female age (model older)	−0.10	−1.68	1.59	0.88
Mate attractiveness (less attractive)	**3.79**	**1.52**	**6.64**	**0.004**
Testing conditions (free-living)	**12.10**	**4.95**	**23.47**	**<0.001**

The analysis included random effects of phylogenetic relatedness, species, and study. Factors considered were mating experience of the observer (virgin vs. non-virgin); age of the model female relative to the observer (whether the female shown associating with a male in the demonstration phase of the experiment was older vs. younger or the same age as the observer); mate attractiveness (whether females were provided with copying information that systematically favored their non-preferred male); and the testing conditions (whether the subjects were captive or free-living during trials). The comparison level of each categorical variable is shown in brackets after the parameter. The effective sample size for each parameter was 1,000. Ninety-five percent CI indicates the 95% credible interval. pMCMC is the Markov Chain Monte Carlo estimate of significance for that effect in the model. Variance due to each random effect is shown in ***Table 1****. Bold text and values indicate statistical significance at* $P < 0.05$.

by <5 are typically interpreted to be equivalent (Spiegelhalter et al., 2014).

Moderators of Mate-Choice copying

Observer Experience

Among the experiments we assessed, observer females that had no previous experience mating (i.e., virgins) were significantly more likely to copy the mate-choice of an observed same-sex conspecific compared to non-virgin individuals ($\Delta\ OR = 1.53$, 95% credible interval = 0.16–2.94, $P = 0.03$, **Table 2**, **Figure 3**, **Figure S3**). When virgin females saw another female choose a male, they were 3.2 times more likely to mate with that male (posterior mean $OR = 3.21$, 95% credible interval = 2.48–4.16). In contrast, females with previous mating experience were 1.7 times more likely to choose a male after he was favored by this type of social information (posterior mean $OR = 1.68$, 95% credible interval = 1.22–2.32).

Model Age

Females were no more likely to copy the mate choice of an older female than that of females their own age ($\Delta\ OR = -0.10$, 95% credible interval = −1.68–1.59, $P = 0.88$, **Table 2**, **Figure 3**).

Male Attractiveness

We found that observer females were more likely to copy the choice of a model female when the model was paired with a less attractive male compared to a random male ($\Delta\ OR = 3.79$, 95% credible interval = 1.52–6.64, $P = 0.004$, **Table 2**, **Figure 3**, **Figure S1**). After females observed a model associating with a randomly chosen male, they were approximately twice as likely to choose that mate in a subsequent trial (posterior mean $OR = 2.13$, 95% credible interval = 1.68–2.71). In contrast, after females saw a model choose a previously non-preferred male, they were nearly six times more likely to mate with that male in subsequent trials (posterior mean $OR = 5.92$, 95% credible interval = 4.22–8.32, **Figure 3**, **Figure S1**). Non-preferred males in this analysis included those identified from known genetic preferences and from pre-tests of observer preference, and so we wondered whether these situations might elicit different degrees of copying. However, a *post-hoc* analysis found no statistical difference in the strength of mate-choice copying between these two groups ($\Delta\ OR = 4.65$, 95% credible interval = –1.67–24.48, $p = 0.25$; $n = 24$ effect sizes from studies where social information favored a non-preferred male).

Testing Conditions

Mate-choice copying had a stronger effect under free-living conditions compared to captive testing of both captive-bred and wild-caught individuals ($\Delta\ OR = 12.10$, 95% credible interval = 4.95–23.47, $P < 0.001$ **Table 2**, **Figure 3**, **Figure S2**). When free-living females saw another female with a male, they were approximately 14 times more likely to subsequently mate with that male (posterior mean $OR = 14.40$, 95% credible interval = 8.28–25.07), while captive females were only 2.3 times more likely to do so (posterior mean $OR = 2.31$, 95% credible interval = 1.60–3.32).

Publication Bias

We found evidence for the presence of publication bias in mate-choice copying studies, as the intercept of a modified Eggers regression was significantly different than zero (intercept + SE: 0.81 + 0.38, $t = 2.11$, $p = 0.037$, **Figure 4**). This indicates that the mean and credible interval of effect sizes may be inflated by the under-reporting of null results. We assessed the impact of this publication bias using the trim and fill test (Duval and Tweedie, 2000), which added 25 data points to our original 103

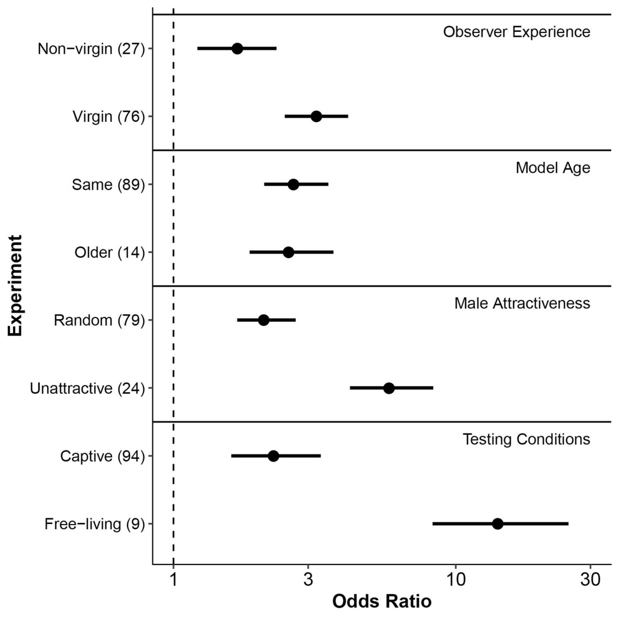

FIGURE 3 | Forest plot of posterior mean effect sizes (odds ratios) of each moderator level hypothesized to affect mate-choice copying. Horizontal lines associated with points indicate 95% credible intervals of the mean odds ratio. Levels of each fixed effect (y-axis) are grouped by moderator type, shown at right. Numbers in parentheses following each level indicate the number of effect sizes included from experiments that met those conditions. The dashed vertical line indicates an odds ratio of 1, equivalent to a 50/50 likelihood of mating with a male after observing another female choose that male, or a phenotypically similar individual. X-axis is on a log10 scale. Model female age refers to the model's age relative to the observer female. In many cases, differences noted referred to relative size rather than age *per se*, as size is a commonly used proxy of age in fish with indeterminate growth.

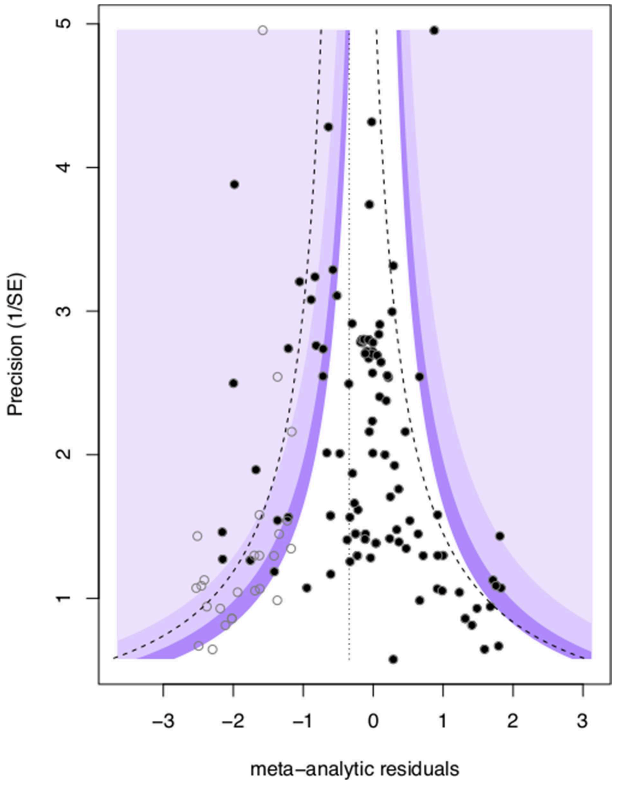

FIGURE 4 | Visualization of publication bias via a contour funnel plot showing meta-analytic residual $ln\ (OR)$ vs. corresponding precision ($1/SE_{ln(OR)}$, black circles). The plot also illustrates the trim and fill "missing" data points (open circles) required to restore funnel symmetry. The dotted line represents the $ln\ (OR)$ adjustment which was applied to the mean of the meta-analytic residuals to correct for publication bias.

and provided a $ln\ (OR)$ estimate adjustment of −0.345. Adjusting our original mean estimates to account for publication bias reduced the size of the mean effect of mate-choice coping, but the effect remained statistically significant ($OR = 1.92$, 95% credible interval 1.13–3.40).

DISCUSSION

Our meta-analysis indicated that female mate-choice copying is a widespread and influential phenomenon. Across all studies assessed, females were on average 2.7 times more likely to choose a particular mate if they received social information favoring that male, compared with females that did not receive such social information. The significant tendency for mate-choice copying to occur remained evident after publication bias was taken into account. We found that mate-choice copying was more likely to occur when the females receiving social information lacked mating experience, or when they received new information about males that were initially "unattractive." However, we found no consistent support for the idea that observer females preferentially attend to the mating decisions of older females. Unexpectedly, there was a strong effect of testing conditions on whether mate-choice copying occurred; effects reported from free-living animals were considerably stronger than those from captive individuals. The prediction interval for the overall effect of mate-choice copying crossed 1 on the OR scale, indicating that evidence for mate-choice copying may not always be present depending on the context and species. In other words, our analysis included some experiments in which mate-choice copying did not occur, and indeed both copying and non-copying strategies have been theoretically predicted to persist in the same population (Dubois et al., 2011). The situations in which mate-choice copying does and does not influence observed mating choice therefore are an interesting area for further research.

As predicted theoretically, we found that females with no mating experience (virgins) were more likely to copy mate choices than were non-virgin females or mature females of unknown sexual history. Social information should logically be most useful to individuals who lack complete information for decision-making, and the disproportionate occurrence of mate-choice copying by virgins supports this assumption. Despite studies supporting the idea that females pay more attention to social information from older individuals (Dugatkin and Godin, 1993; Amlacher and Dugatkin, 2005), we found no overall trend for increased occurrence of mate-choice copying when model females were older than observers. In most cases, model females were age- or size-matched with observer females, and mate-choice copying was as likely to occur in such cases as in situations when the observer was older or larger. However, we did not have enough information to assess other types of asymmetry between the observer and demonstrator females. For example, copying females may attend more to the behavior of familiar, healthy, attractive, or socially dominant conspecifics. In cases where species identity may be questionable, females may attend to information from conspecifics over that from heterospecifics (Hill and Ryan, 2006). These factors were not consistently assessed in the studies we considered here, and so were not included in the current analysis but represent interesting possibilities for future research into how model characteristics may influence observer choice.

Mate attractiveness modified the occurrence of copying. Positive social information had a relatively minor effect on mating behavior when it favored preferred mates, but substantially increased the likelihood that females chose non-preferred males, as was demonstrated in field tests of ocellated wrasse mate choice (Alonzo, 2008). One methodological consideration that could influence the accuracy of our estimate for effects of mate attractiveness is the calculation of null expectation. We classified relative attractiveness of males as whether or not the experiment provided social information that systematically favored the least preferred male. In such situations, the ideal baseline comparison group is a set of females tested to quantify their likelihood of switching mates in a second trial in the absence of social information. We assumed a baseline of 50/50 odds of switching when such information was not available. Females of some taxa have shown a tendency to switch mate choices in repeated trials. For example, a study of zebra finch copying found that females switched to spend more time near a previously non-preferred mate in second trials, whether or not that mate was seen with a model female (Doucet et al., 2004). This effect could lead to a bias in favor of the conclusion that mate-choice copying was taking place. In future work, we recommend that studies investigating mate-choice copying not assume the null result is a 50:50 outcome, but instead explicitly test the baseline probability that females reverse mating preferences when repeatedly exposed to potential mates in the absence of social information.

Of all explanatory factors considered, testing condition had the strongest effect on the occurrence of mate-choice copying. Females were considerably more likely to copy mate choices when experiments tested free-living females rather than those in captivity. The vast majority of studies of mate-choice copying have been conducted in the lab, and reasonably so; it is difficult to control testing conditions of wild animals. There are several reasons why copying may be more prevalent in animals tested in the wild. It is possible that species or populations that are easily tested in the wild also happen to be those that most readily use social information in mate choice. It's also possible that mate-choice copying effects are intensified in situations typical of wild populations. For example, the possible presence of predators could make slower mate choices risky, favoring copying behavior (Gibson and Höglund, 1992). Further, females in the wild may be faced with more mating options compared to the typical dichotomous choice in captivity, and thus rely more on the observed choices of other females to inform their own mating decisions. Similarly, female competition for mates may be stronger in the wild, driving the need to make timely mate-choice decisions. Copying the choice of another female may take less time than acquiring direct information about the quality of a mate. Free-living females may use mate-choice copying more frequently as a time-saving strategy to ensure mating opportunities are not missed. Differences in food availability between lab and field populations may also affect results. However, in one systematic test of this effect, guppies were more likely to copy mate choices when they were satiated, not when hungry as predicted if mate-choice copying reduces decision time (Dugatkin and Godin, 1998). There remains much to be gained both from studies in the wild of species documented to copy mate choices in the lab, and from lab studies that systematically test how environmental factors modify the occurrence of mate-choice copying.

Not unexpectedly, our dataset had a high level of heterogeneity. In the random effects model that accounted for only phylogeny, repeated measures of individual species, and multiple effect sizes derived from the same study, the vast majority (75%) of variance in the data was attributed to differences among species. However, including moderators of observer experience, relative age of the model female, male attractiveness, and test conditions explained species-level variance, indicating differences that appeared to be explained by species identity were more appropriately attributed to combinations of these fixed effects. Likewise, the mixed and random effects models did not differ in their short-term predictive ability (as indicated by their DIC values), but the mixed model indentified factors that explained virtually all the heterogeneity among the species represented in our meta-dataset. However, this pattern furthermore highlights the trend that studies assessing the same species often apply similar testing conditions. For example, all *Drosophila* studies were conducted under laboratory conditions and using virgin focal females. The Trinidadian guppy remains the best-studied species for understanding mate-choice copying and is the only species in our dataset for which effects of all moderators have been investigated.

The reported differences between levels of the explanatory factors are likely underestimated, specifically for male attractiveness and observer experience, as one of the two compared levels for each factor included unavoidable uncertainty. For example, for classification of observer experience, there was no ambiguity when studies used virgin female observers, but when observers were drawn from a population of unknown age or experience (e.g., wild studies, mixed-sex tanks), the testing group almost certainly included a mix of individuals, some of which might have been virgins or females with relatively low sexual experience. Likewise, for tests of mate attractiveness, studies employing experimental reversals were compared those in which researchers assigned a model female to one of two males at random or allowed the model to choose (i.e., non-reversals). Males paired with models in non-reversal studies were not necessarily more attractive than the unpaired males. However, we assumed that they were on average more attractive than paired males in the reversal trails, which were known to have been previously rejected by female observers. We therefore predict that the true difference in effect sizes of the occurrence of mate-choice copying between less attractive and more attractive males is larger than reported.

The phylogenetic signal of the strength of mate-choice copying was virtually non-existent, indicating that this behavior is taxonomically widespread. Likewise, mate-choice copying was not consistent in several closely related species, e.g., there is strong support for the occurrence of mate-choice copying in *Drosophila melanogaster* (Germain et al., 2016; Danchin et al., 2018; Nöbel et al., 2018), but tests in *Drosophila serrata* found no copying (Auld et al., 2009). However, studies that met our criteria of providing individual-level counts of mating choices represented only 17 total species. Several prominent clades for which social learning is evident in other contexts were not represented here, including mammals (Box and Gibson, 1999), amphibians (Ferrari et al., 2007), and cephalopods (Fiorito and Scotto, 1992). This lack can be attributed in part to our exclusion of studies that measured mate choice in a manner other than discrete choices. Conducting an analysis of effect sizes derived from differences in means (e.g., differences in the means of time spent near competing males) would broaden taxonomic representation. However, low taxonomic representation of some clades reflects the lingering lack of tests in those taxa. While it makes sense that highly social animals would be more likely to transfer information among conspecifics during mate-choice, studies robustly indicating the occurrence of mate-choice copying in *Drosophila melanogaster* and in wolf spiders indicate that complex sociality is not required for mate-choice copying to occur, and that a phylogenetic interpretation of the pattern of mate-choice copying across taxa suggests that it occurred in the common ancestor of all Nephrozoa (Fowler-Finn et al., 2015; Danchin et al., 2018; Monier et al., 2018; Nöbel et al., 2018). We suggest that mate-choice copying is not an isolated and discreetly evolved trait within social animals, but rather one of many manifestations of an underlying evolved cognitive ability to observe and copy the choices of others. If mate choice copying results from the same cognitive processes that underlie conspecific cueing in foraging and habitat choices, the cost:benefit ratio of copying may be quite low.

The results of this meta-analysis suggest several areas for future work. First, as noted above, it would be interesting to expand the meta-analysis framework to studies that measured mate-choice copying in different ways. While we restricted our meta-analysis to experiments that quantified the number of individual female choices, there are several valid ways of measuring mate-choice copying (or its assumptions). Most notably, we did not include studies that relied on time spent near a male following or during the presence of a model (e.g., Höglund et al., 1995), though we consider this a valid alternative to quantifying discrete choices. Expanding the types of component analyses included in a meta-analysis of copying effects may allow investigation of hypothesized mate-choice copying effects that we were unable to address here. For example, is mate-choice copying of preference for specific individuals stronger than generalized copying of socially preferred males' phenotypes? Only a few of the studies included here tested for generalized copying behavior rather than preference for the specific individual preferred by other females. These reported that copying was indeed a phenotype-general phenomenon. For example, zebra finches (*Taeniopygia guttata*) copy choices for leg band color in general, not only for individual males (Swaddle et al., 2005). In the dataset used here, we were unable to test whether the occurrence of generalized phenotype copying was robust across studies, or whether the strength of copying effects was weaker (or stronger) in these generalized situations. Additionally, future studies should test the assumption that mate-choice copying decreases costs of independent choice, for example by decreasing decision time during mate choice. We were unable to assess effects of mating system on mate choice copying, as nearly all tested species were socially polygamous. It would be interesting to investigate whether mate-choice copying varies with different types of polygamy, as well-testing for effects in an expanded sample of monogamous species. Finally, several studies noted that the amount and consistency of information can influence the expression of copying behavior. When multiple demonstrators reinforce a non-preferred choice, mate-choice copying is stronger (Dugatkin, 1998; Drullion and Dubois, 2008). Likewise, when social information is inconsistent, females are less likely to copy (Drullion and Dubois, 2008). These effects were not tested broadly enough in our dataset to include as factors in this meta-analysis, but represent interesting avenues for future work.

Not unexpectedly, we detected significant publication bias, especially among studies with relatively small sample sizes. Because mate-choice copying influences mating decisions, it has the potential to influence patterns of sexual selection. Therefore, it is important to understand when copying is and is not a factor. We hope that this report will encourage publication of results rejecting as well as supporting the occurrence of mate-choice copying behavior. Indeed, our results indicate that mate-choice copying should not necessarily always be present (as indicated by the broad 95% prediction interval of the overall effect). The average effect sizes reported here should be useful in power analyses for planning and publishing future work on copying behavior, regardless of the study's outcome.

The support for a robust effect of mate-choice copying on mating decisions across taxa indicates that this phenomenon is a widespread part of mating behavior, particularly in wild systems, though it remains to be incorporated into much current research investigating the process of sexual selection by mate-choice. This meta-analysis identified several contexts—inexperienced observers, information favoring less attractive males, and animals in free-living situations—in which mate-choice copying is more likely to occur, an important step toward predicting its evolutionary consequences (Verzijden et al., 2012). Understanding the strength and moderators of mate-choice copying effects will be an important part of more clearly incorporating this phenomenon into our understanding of social behavior and sexual selection.

AUTHOR CONTRIBUTIONS

Both authors contributed equally to the final manuscript. ED conceived the analysis and planned the conceptual framework. BJ conducted statistical analyses and developed figures. Both authors drafted and edited 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.2019.00390/full#supplementary-material

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Honesty of Larval Begging Signals Covaries With Colony Kin Structure in *Formica* Ants

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Social insects live in highly complex societies with efficient communication systems. Begging is one display commonly used by offspring to signal their nutritional state, however begging behavior has received very little attention in social insects. Theory predicts that begging can be either an honest (i.e., honest-signaling strategy) or a dishonest (i.e., scrambling competition) signal of need, with dishonest signals expected to be more likely when relatedness within the group is low. To investigate the presence and honesty of begging, as well as the nature of the involved signals, we used a comparative approach with four species of the ant genus *Formica* known to differ in the degree of intra-colony relatedness. We investigated the behavior of starved and non-starved larvae of *F. aquilonia*, *F. pressilabris* (both low intra-colony relatedness), *F. exsecta* (intermediate relatedness), and *F. fusca* (high relatedness). In addition, we assessed the attraction of conspecific workers toward odors extracted from these two classes of larvae and analyzed the larval cuticular hydrocarbon profiles. We found that in *F. fusca* and *F. exsecta*, larvae signaled significantly more when starved. In contrast, larvae of *F. aquilonia* signaled significantly more when they were non-starved, while there was no significant difference in the behavior of starved vs. non-starved larvae in *F. pressilabris*. Our results show that workers were not preferentially attracted to the odor of starved larvae, and we also did not detect any differences between the cuticular hydrocarbon profiles of starved and non-starved larvae. Overall, this study demonstrates among species variation in larval hunger signaling in *Formica* ants, and encourages further studies to confirm the link between kin structure variation and the honesty of begging signals.

Keywords: social insects, kin conflict, parent-offspring conflict, honest signal, scramble competition, relatedness, cuticular hydrocarbons

INTRODUCTION

Communication systems allow individuals to base their adaptive decisions on the behavior, morphology, or physiology of others (Endler, 1993). Signals have evolved to convey information to receivers, so that the information extracted from the signal elicits a response and results, on average, in positive fitness consequences for both the sender and the receiver (Laidre and Johnstone, 2013).

To be efficient these interactions must depend on a repertoire of signs and rules shared between the participants of the interaction (Maynard Smith and Harper, 2003; Witzany, 2010). Communication and signaling systems thus allow individuals to affect the plastic decisions of others, possibly generating coevolution (Font and Carazo, 2010).

Despite the social nature of communicative signals, the adaptive function of signals from the receiver's perspective may be fundamentally different from that of the sender (Marler, 1961). Traditionally, communication has been seen as a voluntary transfer of information from the sender to the receiver, when the inclusive fitness interests of the sender and the receiver converge. However, when the inclusive fitness interests of the sender and the receiver diverge, the ultimate aim of the sender is not the sharing of mutual beneficial information, and conflicts over information use arise (Owren and Rendall, 1997, 2001; Seyfarth and Cheney, 2003). In such cases, selection should favor senders, whose signals affect the behavior of the receiver at their own advantage, and receivers, who are able to extract information from the signals (Endler, 1993; Font and Carazo, 2010). Kinship and the inclusive fitness benefits gained through helping relatives (Hamilton, 1964) should influence the type of communication. Closely related individuals should be more likely to communicate honestly in order to cooperate, whereas the opposite would be expected for non-relatives.

Begging is commonly used by offspring to influence the quantity and quality of received food, with consequences for survival (Hodar et al., 2002) and other fitness-related traits (Breteler and Gonzalez, 1988; Chapman, 1998; Shafiei et al., 2001; Engels and Sauer, 2007). Godfray (1991, 1995) developed the idea that begging should be an honest signal of need. Food provisioning by parents is obviously a cooperative behavior but contains an element of conflict as well. This is because the existence of relatedness asymmetries leads offspring to value themselves more than they value their siblings, and thus, to selfishly beg for a larger part of parental investment. However, for a parent, providing one offspring with a larger investment influences the trade-off between the expected fitness returns from this offspring vs. the others (Trivers, 1972, 1974). Therefore, parents would benefit from accurately assessing the level of need expressed through the begging signal. Other models, called scramble competition models, predict that this conflict between parent and offspring over the distribution of resources is increased by competition among siblings (Parker and Macnair, 1979). In such a case, each offspring is assumed to beg more than other offspring even when already fed, leading to an exaggeration of need (Godfray, 1995; Royle et al., 2002; Johnstone, 2004; Akçay, 2012). Scramble competition, and consequently dishonest signaling, should thus be more prevalent when competition occurs among a greater number of siblings or when the relatedness among brood is low (Parker et al., 2002; Johnstone, 2004). This is because the inclusive fitness costs of dishonestly taking food from other brood members are lower under low relatedness. In contrast, signals should be honest indicators of need under high relatedness.

Both honest and dishonest begging has been demonstrated previously. Food-deprived pigeon squabs (*Columba livia*) spend more time begging than recently fed ones (Mondloch, 1995), giving support to honest signaling models. Conversely, begging calls of yellow-headed blackbird chicks varied with the intensity of sibling competition, independent of need (Price et al., 1996). Empirical studies on begging behavior are taxonomically highly biased toward birds and consequently also heavily focused on vocal and visual signals (Kedar et al., 2000; Leonard and Horn, 2005; Marques et al., 2008; Klenova, 2015; Caro et al., 2016). However, offspring of insects with maternal care also display various begging behaviors, mainly in order to solicit protection and food (reviewed in Mas and Kölliker, 2008). For instance, burying beetle larvae (*Nicrophorus*) beg by approaching their parents' mouthparts, pushing their heads against them and touching them with their legs (Milne and Milne, 1976; Rauter and Mooref, 1999).

Colonies of social insects—ants, bees, wasps, and termites—are especially interesting to study from a signaling perspective because they form large, complex societies, which function based on chemical, visual, acoustic, vibrational, and tactile signals (Leonhardt et al., 2016). In ants, kinship among colony members varies widely due to variation in queen number and queen mating frequency. This makes them particularly suitable for testing predictions concerning signaling honesty. Recently, selfish brood behaviors were shown to be correlated with low relatedness, raising the possibility that brood might indeed respond to kin structure variation in adaptive ways (Schultner et al., 2013, 2014). However, offspring begging as a classic conflict-related trait has been largely overlooked in social insects. The few empirical studies that have characterized begging in social Hymenopteran larvae show that various behavioral hunger signals exist, from scraping mandibles against cell walls (vespine wasp larvae: Ishay and Schwartz, 1973) to flexing the head or whole body to attract workers (ant larvae: Brian, 1977; Creemers et al., 2003; Kaptein et al., 2005). For example, *Gnamptogenys striatula* ant larvae perform two types of movement to signal their hunger toward workers after a 24 h starvation period: "swaying," which corresponds to a repositioning of the larval body to reach food or workers and "stretching," which corresponds to a rhythmic stretching of the body (Kaptein et al., 2005). Hungry larvae perform these movements more often, indicating that begging signals honestly reflect need in this species. Similar behavioral patterns have been detected in *Myrmica* ant larvae (Creemers et al., 2003). Additionally, larvae may solicit food from workers using chemical hunger signals in bees (den Boer and Duchateau, 2006; He et al., 2016), and fire ants (Cassill and Tschinkel, 1995). While these studies provide first evidence for the ability of social insect larvae to influence their food intake via begging, comparative studies are needed to test how begging in social insects is influenced by colony kin structure.

We used a comparative approach to investigate larval hunger signaling in ants. Our aim was to assess the existence of hunger signals and the effect of kinship on the honesty of these signals. We focused on *Formica* ants because their colony kin structures range from single queen colonies with high intra-colony relatedness to multiple queen colonies with extremely low relatedness among nestmates (Rosengren et al., 1993; Sundström et al., 2005). For four species of *Formica* ants, representing a range of intra-colonial relatedness, we assessed the behavior of starved and non-starved larvae. We furthermore analyzed the cuticular odors of starved and non-starved larvae and experimentally tested for potential chemical hunger signals by presenting workers with larval cuticular extracts. In line with inclusive fitness predictions, we find that starved larvae signaled significantly more than non-starved larvae in *Formica fusca* and *F. exsecta*, i.e., species with high and intermediate levels of intra-colony relatedness. In contrast in *F. aquilonia*, a low relatedness species, larvae signaled significantly more when they were not starved, while there was no significant difference in the behavior of starved vs. non-starved larvae in the other low relatedness species. Workers were not preferentially attracted to the odor of starved larvae, and we also did not detect any differences between the cuticular hydrocarbon profiles of starved and non-starved larvae. Our study demonstrates that larval hunger signaling varies among species in ants and suggests links between kinship and the honesty of hunger signaling in social insects.

MATERIALS AND METHODS

Study Species and Experimental Setup

Formica ants are characterized by remarkable intra- and interspecific variability in within-nest relatedness, largely due to variation in queen numbers (Rosengren et al., 1993; Sundström et al., 2005). We studied four *Formica* species covering the full range of relatedness values: *F. aquilonia* forms large networks of interconnected nests (so-called polydomy), where each nest contains hundreds of queens (Rosengren et al., 1993; personal observation), and relatedness among nestmates is very low ($r = 0.06 \pm 0.09$ CI, Schultner et al., 2014, 2016). *F. pressilabris* is also typically polydomous and nests contain multiple queens, usually some dozens ($r = 0.21 \pm 0.09$ CI, Schultner et al., 2014). These species are therefore classified as "low-relatedness" species. In contrast, *F. fusca* nests typically contain ∼1–10 queens (Hannonen and Sundström, 2003; Helanterä et al., 2011), and each colony comprises one nest only (so-called monodomy). This species is thus considered a "high-relatedness" species ($r = 0.46 \pm 0.14$ CI, Schultner et al., 2014). *F. exsecta* can form both monodomous colonies containing single queens and polydomous colonies in which each nest contains dozens of queens (Rosengren et al., 1993). The *F. exsecta* colonies used in this study were collected from a polydomous population ($r = 0.09 \pm 0.08$ CI, Schultner et al., 2014). However, in our study area most nests of this species form monodomous populations, in which colonies have only one queen and very high relatedness ($r = 0.71 \pm 0.03$ SE; Sundström et al., 2003). Compared to *F. aquilonia* and *F. pressilabris*, which predominantly live in polydomous, low relatedness populations, and *F. fusca*, which always live in monodomous, high relatedness populations, *F. exsecta* is therefore considered an "intermediate relatedness" species. Note that of the four species, only *F. fusca* founds colonies independently, while the other three species are so-called temporary social parasites, which found colonies in the nests of other ants, including *F. fusca* (Buschinger, 2009), or dependently with the help of conspecific workers. All colonies used in this study were collected from established populations in which the host species have already been eradicated, and none contained any host workers.

From each species, colony fragments including queens, workers, and nest material were collected in the field and brought back to the laboratory. Colony fragments were then placed in containers lined with Fluon, together with a ceramic plate, some compost as nest material and *Sphagnum* moss to maintain humidity. The colonies were fed with Bhatkar-Whitcomb diet (Bhatkar and Whitcomb, 1970) and watered daily. Colony fragments were checked daily for the presence of eggs. When the first eggs were observed, up to 10 queens were taken from each fragment, placed individually in Petri dishes with two pieces of 1 cm × 1 cm humid sponge and kept in the dark at room temperature. After 2 days, queens were removed and put back into their colony fragments. Eggs deposited on the Petri dishes were then counted and checked every 2 days to remove fungal spores, re-humidify sponges and remove dead eggs. Eggs from all species hatched within 9–15 days.

We created two groups of larvae differing in regard to their state of hunger for behavioral observations and chemical assays. Freshly hatched larvae (0–3 h old) were considered not to be hungry ("non-starved" hereafter), and were either moved to a new Petri dish, in which their behavior was observed immediately (see "Quantification of larval behavior"), or directly collected in glass vials for chemical analyses (**Figure 1**). To obtain hungry ("starved" hereafter) larvae, freshly hatched larvae were moved to a new Petri dish containing a piece of 1 cm × 1 cm sponge, where they remained for 21–24 h. Larvae were thereafter subjected to behavioral or chemical analyses as described for non-starved larvae (**Figure 1**). Larvae did not have the opportunity to cannibalize one another on the Petri dish because they were physically separated, and the total number of larvae on each Petri dish was documented. No larvae disappeared from any of the Petri dishes during the starvation period, indicating that cannibalism did not occur. The number of replicates conducted for behavioral assays and the number of samples used in chemical analyses are summarized in **Table 1**.

Quantification of Larval Behavior

To quantify begging behavior, groups of 2–5 starved or non-starved larvae were placed on their backs in a Petri dish (**Figure 1**). The Petri dish was put under a stereomicroscope onto which a camera (DigiMicroScale) was installed and behaviors were recorded for 10 min. Each group consisted only of larvae produced by the same queen, thus groups were genetically homogenous. To avoid any matriline bias, several groups of larvae produced by different queens from each colony were used (at least two different queens). To avoid observer bias, videos were renamed by someone unfamiliar with the experimental setup prior to analysis. We observed two different kind of behaviors performed by the larvae. The first one, termed "swaying" in reference to Kaptein et al. (2005), consisted of the larva moving its body upwards, remaining in this position for some time and/or rhythmically repeating the movement. The second recorded behavior was a rhythmic opening and closing of the mandibles. This behavior has been implicated in larval begging in attine ants (Wheeler and Bailey, 1920),

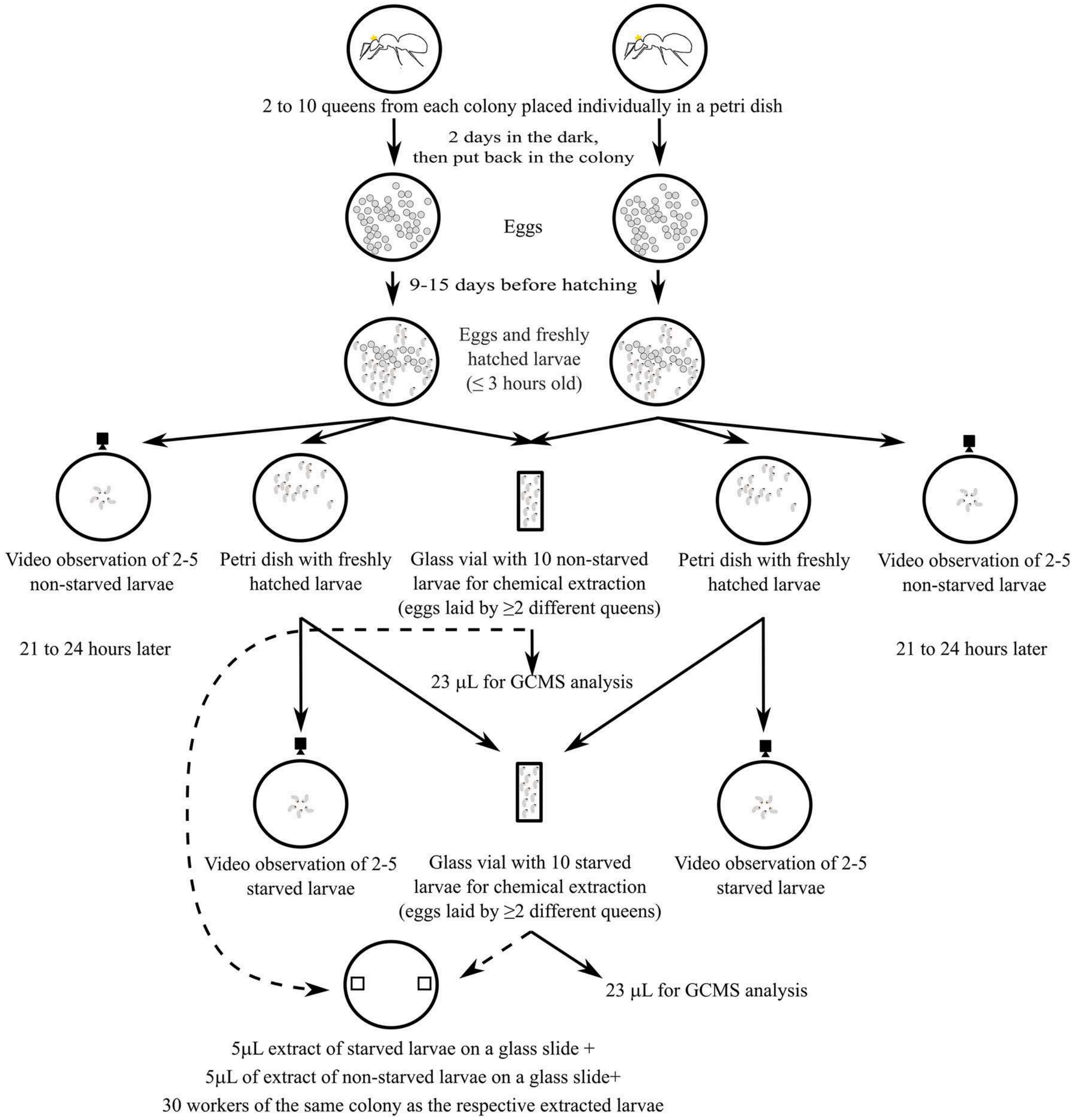

FIGURE 1 | Experimental setup for rearing of larvae of one species, the same setup was used for all the species. Eggs are represented by gray dots. Non-starved larvae hatched between 0 and 3 h after the first observation. Starved larvae hatched between 0 and 3 h after the first observation and were placed on a separate Petri dish for an additional 21–24 h. All the video observations have been carried out with larvae from a single matriline.

and preliminary observations suggest it may correlate with nutritional status in Formica ants. For each larva, the number and duration (in seconds) of swaying movements and the number and duration of mandible movements were recorded with the software Boris (version 6.3.1, Friard and Gamba, 2016).

Intracolony Relatedness and Larval Sex Ratios

Levels of selfishness–in the form of egg cannibalism–exhibited by *Formica* ant larvae depend on intra-colony relatedness and larval sex (Schultner et al., 2013, 2014). While female larvae behave less selfishly when within-colony relatedness is high, male larvae do not adjust their levels of selfishness to variation in colony kin structure. In order to estimate the effect of intra-colony relatedness and larval sex on begging behavior, we determined relatedness and sex of larvae from genotype data using six microsatellite loci previously tested in our focal species (FE 19, FE 42, FE 51, FY 4, FY7, FL20; Schultner et al., 2013, 2014, 2016). To this end, we collected 2–7 larvae from 10 to 15 colonies of each species in 70% EtOH (**Table 1**). For DNA extraction, each larva was placed in an individual well with a 2.5 µl: 100 µl Proteinase K–Chelex solution (6%), and incubated overnight at 56°C. After inhibition of Proteinase K activity at 100°C for 10 min, 1 µl of DNA was used in simplex PCR reactions, together

TABLE 1 | Number of larvae, workers, and colonies used in behavioral assays and for chemical and genetic analyses.

Experiment species		Starved treatment		Non-starved treatment	
Behavioral assays		**Replicates**	**Colonies**	**Replicates**	**Colonies**
Larval begging behavior	*F. fusca*	131	14	41	8
	F. exsecta	86	10	43	7
	F. pressilabris	92	10	80	9
	F. aquilonia	100	10	68	8
Worker response to larval extracts	*F. fusca*	135	15	135	15
	F. exsecta	72	8	72	8
	F. pressilabris	108	12	108	12
	F. aquilonia	108	12	108	12
Chemical analysis		**Colonies**		**Colonies**	
Larval cuticular hydrocarbon profiles	*F. fusca*	7		8	
	F. exsecta	3		2	
	F. pressilabris	7		8	
	F. aquilonia	11		10	
Genetic analysis		**Replicates**		**Colonies**	
Larval genotypes	*F. fusca*	66		15	
	F. exsecta	41		10	
	F. pressilabris	48		10	
	F. aquilonia	56		9	

For larval begging behavior, the replicates represent the number of individual larvae whose behavior was recorded (when we did not exclude the 5 colonies with species-level values of relatedness and sex ratio, see Statistical Analyses). For worker response to larval extracts, the replicates represent the number of data points collected for each species (e.g., the number of colonies used in a species multiplied by 9, the number of hours during which we extracted the maximum number of workers on each glass slide). For chemical analysis, each colony represents one replicate of a pool of 10 larvae. For genetic analyses, larvae were collected randomly from starved and non-starved treatments; each replicate corresponds to one larva.

with 5 μl of TAQ polymerase, 3 μl of H_2O, and 1 μl primer (0.5 μl forward primer, 0.5 μl reverse primer). PCRs were run with protocols optimized for each primer and PCR products analyzed in a 310 ABI PRISM sequencer (Applied Biosystems). Each microsatellite peak was scored manually with GeneScan 3.1 software (Applied Biosystems).

Intra-colony relatedness was estimated with Relatedness 5.0.8 software (Queller and Goodnight, 1989) using background allele frequencies calculated from the genotypes generated for this study. For all relatedness estimates, standard errors were calculated by jackknifing over colonies. To determine larval sex, individuals were scored as females if they were heterozygous at one or more loci and as males if they were homozygous at all six loci. In some cases, not all loci amplified successfully; for putative males, which were homozygous at <6 loci (15 out of 29 homozygous individuals), we calculated the probability of wrongly assigning a larva as a male using previously established methods (Schultner et al., 2014), and scored larvae with a misclassification probability below 10% as males (0 putative male larvae excluded). For each colony, the average larval sex ratio was calculated from sexed individuals, with $0 =$ all females and $1 =$ all males.

Worker Response to Larval Odors

From several (at least two) queens from each colony fragment, we collected and froze pools of 10 starved and 10 non-starved larvae in individual glass vials (Sigma Aldrich) (**Table 1**). Cuticular compounds were extracted from each group of 10 larvae for 1 min with 30 μL of hexane. Thereafter, 28 μl of the solvent were transferred to a new, clean vial. From each pooled sample (e.g., each group of 10 larvae), 5 μl were used to investigate chemical hunger signaling. The test setup consisted of two 0.5 cm × 0.5 cm glass slides that were placed in a round container (7 cm diameter). The containers' sides were covered with black paper and the containers themselves were covered with red transparent paper and placed in a cardboard box to minimize luminosity and disturbance. In each container, one glass slide was covered with 5 μL of cuticular extract from non-starved larvae (hereafter, "non-starved odor") while the other one was covered with 5 μL of cuticular extract from starved larvae (hereafter, "starved odor") (**Figure 1**). After the solvent had evaporated (30 min), 30 workers were added from the same colony fragment as the respective extracted larvae. After 30 min of adaptation, 10 pictures were taken at 5 s intervals at every full hour for 9 h, for a total of 90 pictures per container. Each camera (two in total, camera model: SONY HDR-SR8E) was able to capture 12 containers at the same time. The number of workers with at least one body part (legs, antennae) on the glass slide was recorded in each picture for each glass slide. To avoid any side bias, the position of the cuticular extracts was randomized among replicates for each species. Prior to analyses, the pictures were renamed by someone unfamiliar with the experimental setup to avoid any observer bias. We predict that if a chemical signal is produced by larvae to signal their state, workers should be more attracted toward the odors of starved larvae than to the odors of non-starved larvae, irrespective of the species.

Characterization of Larval Chemical Profiles

Chemical analyses were conducted on the remaining 23 μl of each sample. Samples were fractionated on silica columns (Chromabond SiOH, 1 mL/100 mg) to remove fatty acids (likely originating internally, as the larval cuticle is thin and not all larvae remained intact throughout the extraction). The non-polar hexane fraction was carefully evaporated under a stream of nitrogen, and then re-dissolved in 23 μl of hexane. Two μl per sample were then subjected to gas chromatography with coupled mass spectrometry (GCMS, Shimadzu GCMS-QP2010 Plus) fitted with a SGE BPX-5 column (31 m × 0.25 μm × 0.25 mm). Helium gas (1 mL/min) served as carrier gas, and injection was splitless. The temperature program started at 70°C isothermal for 1 min, after which the temperature was raised to 200° C at 30°C/min and then from 200 to 320°C at 5°C/min, where it was held for 5 min. As larval samples showed a number of unusual peaks, we also ran control samples of Petri dishes, which were considered a likely cause of contaminations. For that purpose, four clean Petri dishes were separately rinsed with 30 μL of hexane for 1 min each and the resulting samples were analyzed following the protocol above. The chromatograms of each sample were analyzed using ChemStation software (E.02.01.1177). Compounds detected in samples of the clean petri dishes were also found in larval samples and were thus excluded from further analyses. Tentative identification of the remaining peaks was based on their retention indices and mass spectra. We used letters to indicate different but unidentifiable peaks, and "x" and "y" to indicate different positions of double-bonds or methyl groups (**Supplement Table 1**). Relative amounts for each of the remaining peaks were calculated following Aitchison (1982):

$$\ln \frac{\text{area of compound A of individual } L_x}{\text{geometric mean of all compound areas of individual } L_x}$$

Statistical Analyses

We conducted all statistical analyses with R software 3.5.1 (R Core Team, 2018). To investigate larval begging behavior, we ran generalized linear mixed models that account for zero-inflated data following a Poisson distribution (function glmmTMB in R package glmmTMB, Brooks et al., 2017). Each of the four measures of begging intensity (number of swaying movements, swaying duration, number of mandible movements, mandible movement duration) were treated as dependent variables and investigated separately. To test for overall differences in begging intensity between species, we constructed two global models. The first model included the effects of species and the interaction between mean intra-colony relatedness and larval state as fixed terms, and the group of larvae observed at the same time as a random term:

$$\text{Global model 1}: \quad \textit{Measure of begging intensity} \sim \textit{relatedness}{*}\textit{state} + \textit{species} + (1|\textit{group})$$

The second global model tested the effects of species and the interaction between mean intra-colony sex ratio and larval state as fixed terms, and the group of larvae observed at the same time as a random term:

$$\text{Global model 2}: \quad \textit{Measure of begging intensity} \sim \textit{sex ratio}{*}\textit{state} + \textit{species} + (1|\textit{group})$$

For global models, overall p-values for each term were obtained using Type II Wald X2 tests (R function anova). For each species, we furthermore constructed two species-specific models. The first species-specific model tested the effect of larval state and its interaction with mean intra-colony relatedness (fixed terms), while the group of larvae observed at the same time was used as a random term:

$$\text{Species} - \text{specific model 1}: \quad \textit{Measure of begging intensity} \sim \textit{relatedness}{*}\textit{state} + (1|\textit{group})$$

The second species-specific model tested the effect of larval state and its interaction with mean intra-colony sex ratio (fixed terms), while the group of larvae observed at the same time was used as a random term.

$$\text{Species} - \text{specific model 2}: \quad \textit{Measure of begging intensity} \sim \textit{sex ratio}{*}\textit{state} + (1|\textit{group})$$

The effects of mean intra-colony relatedness and mean intra-colony sex ratios were tested separately because these factors covary for each individual colony as they are based on one set of genotypes per colony. For five colonies, larval genotypes were not available (*F. aquilonia* = 1 colony, *F. exsecta* = 1, *F. fusca* = 2, *F. pressilabris* = 1); here we substituted species-level mean relatedness and sex ratio values. We ran the two global models and the eight species-specific models without these five colonies to verify whether the results stayed the same. As there were no major differences in the statistical results between the two data sets (compare **Tables 2**, **3**, **Supplement Tables 2–4** and **Supplement Tables 5–9**), we retained the entire data set for all analyses given in the main text.

To compare intra-colony relatedness and sex ratio levels across species, we used Kruskal-Wallis rank sum tests for non-parametric data (R function kruskal.test).

To investigate worker responses to larval odors, we performed generalized linear mixed effects models following a Poisson distribution (function glmer in R package lme4, Bates et al., 2014). We determined the maximum number of workers on a glass slide in each treatment (i.e., starved or non-starved larvae odor) in the 10 pictures from each hour and used it as a response variable. For all species combined, we constructed a model where treatment and species were included as fixed terms while the hour when the picture was taken and the colony from which larvae and workers were sampled were used as random terms:

$$\text{Global model 3}: \quad \textit{Maximum number of workers} \sim \textit{treatment} + \textit{species} + \left(1\middle|\textit{hour}\right) + (1|\textit{colony})$$

We also constructed a separate model for each species, where treatment was used as a fixed term and the hour when the picture was taken and the colony from which larvae and workers were sampled were used as random terms:

$$\text{Species} - \text{specific model 3}: \quad \textit{Maximum number of workers} \sim \textit{treatment} + \left(1\middle|\textit{hour}\right) + (1|\textit{colony})$$

To characterize the chemical profiles of starved and non-starved larvae we performed principal component analyses (PCA) on transformed proportions of compounds (function fviz_pca in R package factoextra). We performed four separate PCAs using the proportions of all compounds, only alkanes, only methylated alkanes or only alkenes. Each of these PCAs was performed on the combined data from all the species, and for each species separately. The 95% confidence ellipses for each species and treatment were calculated by bootstrapping based on the function coord.ellipse in R package FactoMineR (Lê et al., 2008).

TABLE 2 | Results of Generalized Linear Mixed Models using Template Model Builder linking the number of swaying movements, swaying duration, number of mandible movements, or mandible movement duration to the state and species of the larvae, intra-colony relatedness, and intra-colony sex ratio.

	~Relatedness*state + species + (1|group)				**~Sex ratio*state + species + (1|group)**			
	Effect	**Chisq**	**df**	***p*-value**	**Effect**	**Chisq**	**df**	***p*-value**
Number of swaying movements (*n* = 641)	Relatedness	2.009	1	0.156	Sex ratio	0.946	1	0.331
	State	0.912	1	0.340	State	1.091	1	0.296
	Species	0.776	3	0.855	Species	0.55	3	0.908
	Relatedness:state	2.246	1	0.134	Sex ratio:state	9.091	1	**0.003**
	Random variable	Var ± SD			Random variable	Var ± SD		
	(1|group)	0.76 ± 0.87			(1|group)	0.43 ± 0.66		
Swaying duration (*n* = 641)	Relatedness	2.928	1	0.087	Sex ratio	1.616	1	0.204
	State	17.901	1	**<0.001**	State	18.383	1	**<0.001**
	Species	11.400	3	**0.0097**	Species	11.175	3	**0.011**
	Relatedness:state	0.568	1	0.451	Sex ratio:state	2.164	1	0.141
	Random variable	Var ± SD			Random variable	Var ± SD		
	(1|group)	36.54 ± 6.05			(1|group)	36.86 ± 6.07		
Number of mandible movements (*n* = 641)	Relatedness	2.816	1	0.093	Sex ratio	1.118	1	0.290
	State	0.130	1	0.718	State	0.184	1	0.668
	Species	12.379	3	**0.006**	Species	11.088	3	**0.011**
	Relatedness:state	5.360	1	**0.021**	Sex ratio:state	1.503	1	0.220
	Random variable	Var ± SD			Random variable	Var ± SD		
	(1|group)	0.35 ± 0.59			(1|group)	0.37 ± 0.61		
Mandible movement duration (*n* = 641)	Relatedness	0.405	1	0.524	Sex ratio	0.186	1	0.666
	State	2.096	1	0.148	State	7.359	1	**0.007**
	Species	11.202	3	**0.011**	Species	7.81	3	**0.050**
	Relatedness:state	6.471	1	**0.011**	Sex ratio:state	0.0001	1	0.992
	Random variable	Var ± SD			Random variable	Var ± SD		
	(1|group)	1.16 ± 1.08			(1|group)	19.26 ± 4.39		

Significant p-values are written in bold. n represents the number of starved and non-starved larvae used in each model.

RESULTS

Quantification of Larval Behavior

Overall, larvae swayed slightly less often when they were starved, with the number of swaying events averaging 0.47 ± 0.05 SE in starved larvae and 0.58 ± 0.14 SE in non-starved larvae. Swaying duration was also shorter in starved larvae (12.05 s ± 14.55 SE) than in non-starved larvae (12.85 ± 32.29 SE). Similarly, starved larvae moved their mandibles less often (starved: 1.01 ± 0.08 SE; non-starved: 1.06 ± 0.11 SE) and for shorter periods of time (starved: 24.93 s ± 29.67 SE; non-starved: 25.94 ± 46.70 SE). However, only swaying duration differed significantly between starved and non-starved larvae (**Table 2**, fixed term state in global model "Swaying duration," $\chi^2 = 17.90$, $p < 0.001$). Swaying duration, the number of mandible movements and mandible movement duration also differed significantly between species, with *F. aquilonia* larvae signaling significantly more than larvae of the other three species (**Table 2**, Global models "Swaying duration," "Number of mandible movements," "Mandible movement duration").

In contrast, there were no significant effects of intra-colony relatedness or sex ratio on the number of swaying events, swaying duration, number of mandible movements or mandible movement duration (**Table 2**). However, we did find a significant negative effect of the interaction between intra-colony relatedness and larval state on the number and duration of mandible movements (**Figure 2**, **Table 2**, fixed term relatedness*state in global models "Number of mandible movements": $\chi^2 = 5.36$, $p = 0.021$, "Mandible movement duration"; $\chi^2 = 6.47$, $p = 0.011$), indicating that the number and duration of mandible movements decreased significantly with increasing intra-colony relatedness in non-starved but not starved larvae. Additionally, the interaction between intra-colony sex ratio and state had a significant positive effect on the number of swaying events (**Figure 2**, **Table 2**, fixed term state*sex ratio in global model "Number of swaying movements," $\chi^2 = 9.09$, $p = 0.003$). This suggests that the number of swaying movements increased significantly faster with increasing male bias in non-starved compared to starved larvae.

Within-species analyses showed that *F. fusca* and *F. exsecta* larvae swayed more often when starved (**Figure 3**, **Table 3**, fixed term state, *F. fusca*: $z = 2.69$, $p = 0.007$; *F. exsecta*: $z = 2.39$, $p = 0.017$), while *F. aquilonia* larvae swayed more when not starved (**Figure 3**, **Table 3**, fixed term state, *F. aquilonia*: $z = -2.84$, $p = 0.005$). The number of swaying movements performed by *F. pressilabris* larvae did not vary depending on nutritional state (**Figure 3**, **Table 3**, fixed term state, *F. pressilabris*: $z = -0.20$, $p =$

TABLE 3 | Results of Generalized Linear Mixed Models using Template Model Builder linking the number of swaying movements to the state of the larvae, intra-colony relatedness, and intra-colony sex ratio for each species separately.

Number of swaying movements	**~Relatedness*state + (1|group)**				**~Sex ratio*state + (1|group)**			
	Effect	**β ± SE**	***z***	***p*-value**	**Effect**	**β ± *SE***	***z***	***p*-value**
F. fusca (*n* = 172)	Relatedness	1.78 ± 1.95	0.92	0.360	Sex ratio	0.32 ± 4.11	0.08	0.938
	State	1.60±0.60	2.69	**0.007**	State	1.38 ± 0.60	2.29	**0.022**
	Relatedness:state	−0.73 ± 2.02	−0.36	0.716	Sex ratio:state	1.21 ± 4.18	0.29	0.772
	Random variable	Var ± SD			Random variable	Var ± SD		
	(1|group)	0.02 ± 0.14			(1|group)	$4.31*10^{-8} \pm 2.08*10^{-4}$		
F. exsecta (*n* = 129)	Relatedness	−1.29 ± 5.44	−0.24	0.813	Sex ratio	3.89 ± 5.21	0.75	0.456
	State	1.60 ± 0.67	2.39	**0.017**	State	2.00 ± 0.78	2.55	**0.011**
	Relatedness:state	−2.37 ± 5.73	−0.41	0.679	Sex ratio:state	−8.40 ± 5.81	−1.45	0.148
	Random variable	Var ± SD			Random variable	Var ± SD		
	(1|group)	1.08 ± 1.04			(1|group)	1.28 ± 1.13		
F. pressilabris (*n* = 172)	Relatedness	−1.07 ± 1.22	−0.87	0.384	Sex ratio	0.29 ± 1.88	0.15	0.879
	State	−0.06 ± 0.30	−0.20	0.840	State	−0.10 ± 0.37	−0.28	0.779
	Relatedness:state	−0.69 ± 1.64	−0.42	0.674	Sex ratio:state	0.87 ± 2.64	0.33	0.741
	Random variable	Var ± SD			Random variable	Var ± SD		
	(1|group)	0.12 ± 0.34			(1|group)	0.06 ± 0.24		
F. aquilonia (*n* = 168)	Relatedness	−5.27 ± 1.82	−2.89	**0.004**	Sex ratio	7.46 ± 1.44	5.19	**<0.001**
	State	−1.51 ± 0.53	−2.84	**0.005**	State	0.09 ± 0.47	0.20	0.845
	Relatedness:state	4.21 ± 2.31	1.83	0.068	Sex ratio:state	−8.07 ± 1.95	−4.13	**<0.001**
	Random variable	Var ± SD			Random variable	Var ± SD		
	(1|group)	0.82 ± 0.91			(1|group)	0.11 ± 0.33		

Significant p-values are written in bold. n represents the number of starved and non-starved larvae used in each model.

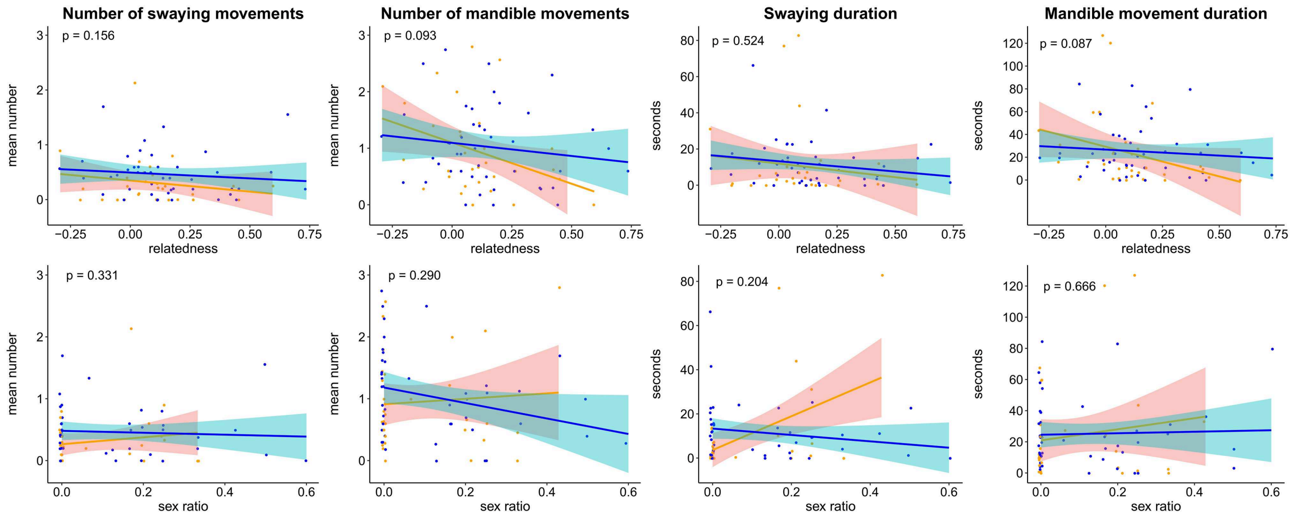

FIGURE 2 | Influence of intra-colony relatedness (top row) and intra-colony sex ratio (bottom row) on the mean number of swaying movements, mean duration of swaying movements, mean number of mandible movements, and mean duration of mandible movements performed by non-starved (orange) and starved (blue) *Formica* larvae. Each point represents the mean from one colony. Regression lines and confidence intervals were drawn using the R function geom_smooth (method = glm). *P*-values denote the results from zero-inflated GLMMs (see **Table 2**).

0.840). There was no difference between starved and non-starved larvae in the other three measures of begging intensity, with the exception of swaying duration in *F. exsecta* (**Supplement Table 2**, fixed term state, *F. exsecta*: $z = 4.72$, $p < 0.001$) and number of mandible movements in *F. aquilonia* (**Supplement Table 3**, fixed term state, *F. aquilonia*: $z = -2.90$, $p = 0.004$). The effect of

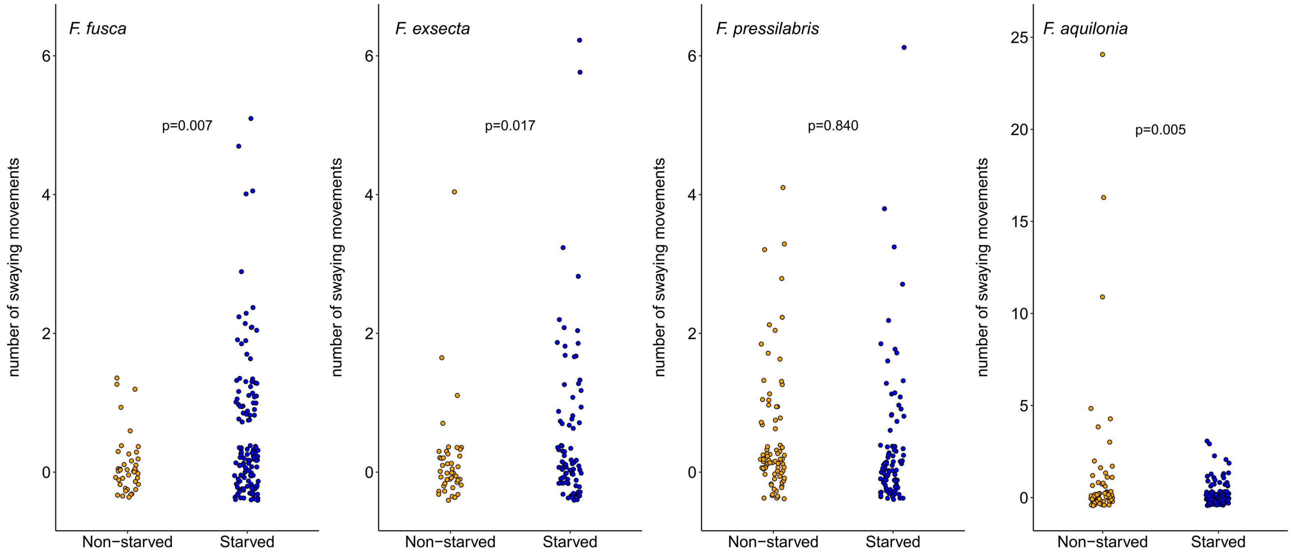

FIGURE 3 | Number of swaying movements performed by non-starved and starved larvae of *F. fusca*, *F. exsecta*, *F. pressilabris*, and *F. aquilonia*. Each dot represents the number of swaying movements performed by one larva. *P*-values denote the results from zero-inflated GLMMs (see **Table 3**). Note different scales on the y-axes.

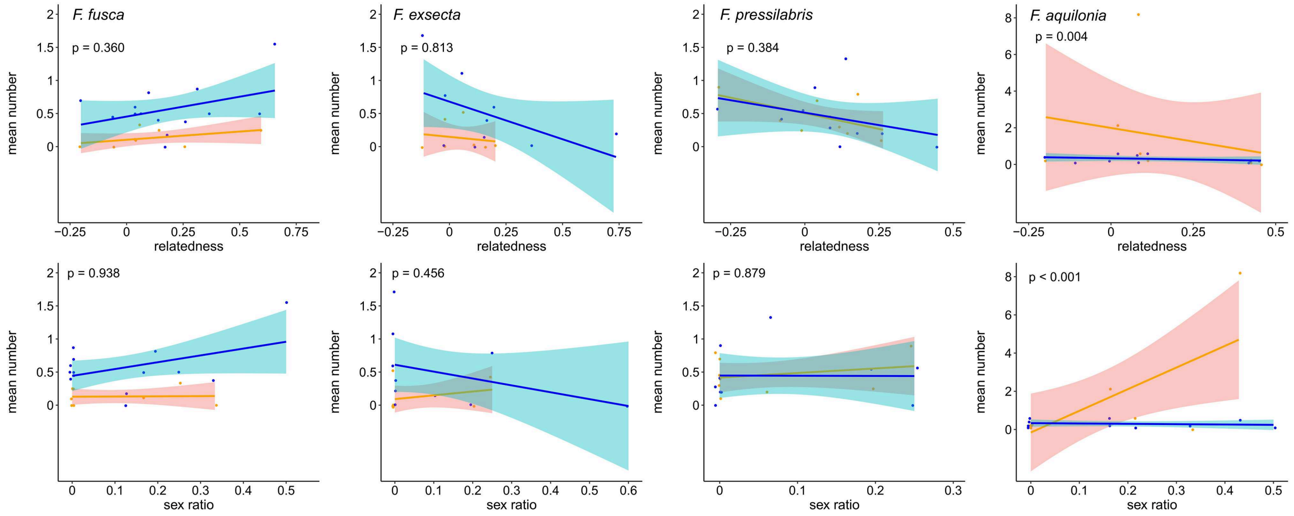

FIGURE 4 | Influence of intra-colony relatedness (top row) and intra-colony sex ratio (bottom row) on the mean number of swaying movements performed by non-starved (orange) and starved (blue) larvae of *F. fusca, F. exsecta, F. pressilabris*, and *F. aquilonia*. Each point represents the mean from one colony. Regression lines and confidence intervals were drawn using the R function geom_smooth (method = glm). *P*-values denote the results from zero-inflated GLMMs (see **Table 3**). Note different scales on the y-axes.

larval state on these behaviors was the same as for the number of swaying movements, with *F. exsecta* larvae swaying longer when starved and *F. aquilonia* larvae exhibiting more mandible movements when non-starved.

Analyses of begging behavior within species furthermore revealed that variation in intra-colony relatedness and sex ratio do not influence begging signals to the same degree in all species (**Figure 4**, **Table 3**, **Supplement Tables 2–4**). For example, increasing relatedness had significant negative effects while increasing male-bias in sex ratio had significant positive effects on the number of swaying movements in *F. aquilonia* (**Figure 4**, **Table 3**, fixed term relatedness, *F. aquilonia*: $z = -2.89$, $p = 0.004$; fixed term sex ratio, *F. aquilonia*: $z = 5.19$, $p < 0.001$) but not the other three species. Conversely, increasing relatedness had significant negative effects on the number of mandible movements performed by larvae in *F. fusca*, *F. pressilabris*, and *F. aquilonia*, but not *F. exsecta* (**Supplement Table 3**, fixed term relatedness, *F. fusca*: $z = -2.14$, $p = 0.033$; *F. pressilabris*: $z = -2.31$, $p = 0.021$; *F. aquilonia*: $z = -2.42$, $p = 0.007$; *F. exsecta*: $z = 4.02$, $p = 0.091$).

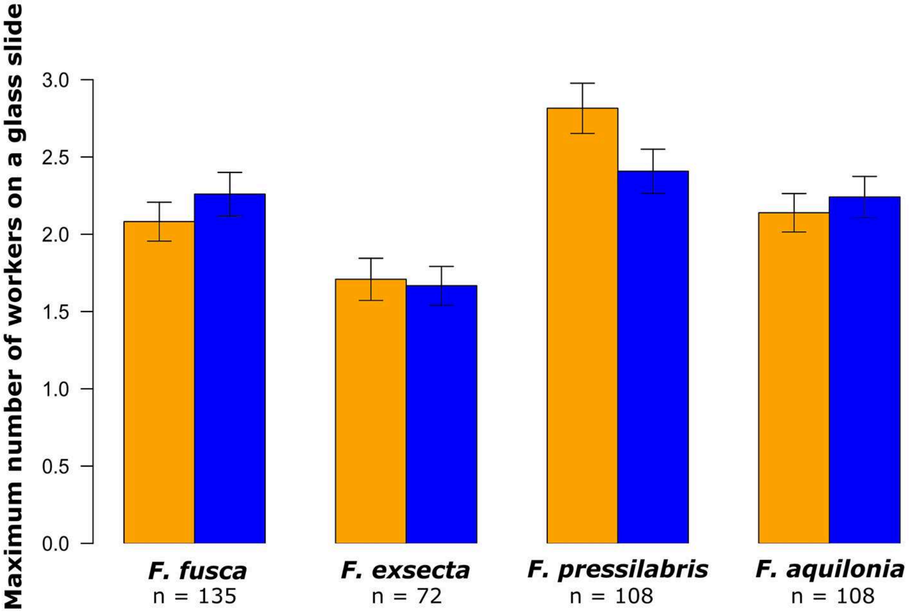

FIGURE 5 | Maximum hourly number of *F. fusca*, *F. exsecta*, *F. pressilabris*, and *F. aquilonia* workers on glass slides covered with odors from non-starved (orange) and starved (blue) larvae. The number of replicates is noted below each species name. The error bars refer to the 95% confidence interval of the mean.

Intra-colony Relatedness and Larval Sex Ratios

F. fusca had a mean intra-colony relatedness of 0.18 ± 0.05 SE and an intra-colony sex ratio (proportion of males) of 0.13 ± 0.19 SD. *F. exsecta* had a mean intra-colony relatedness of 0.16 ± 0.06 SE and an intra-colony sex ratio of 0.11 ± 0.20 SD. *F. pressilabris* had a mean intra-colony relatedness of 0.14 ± 0.07 SE and an intra-colony sex ratio of 0.06 ± 0.11 SD. *F. aquilonia* had a mean intra-colony relatedness of 0.09 ± 0.05 SE and intra-colony sex ratio of 0.21 ± 0.22 SD. There were no differences in intra-colony sex ratios between species (Kruskal Wallis rank sum test, $X^2 = 3.357$, $p = 0.339$). Similarly, intra-colony relatedness estimates showed large overlap and there were no differences in relatedness estimates between species (Kruskal Wallis rank sum test, $X^2 = 0.033$, $p = 0.998$). Intra-colony relatedness was nevertheless included as an explanatory variable in the statistical analyses of begging intensity in order to test whether begging behavior is plastic, i.e., larvae adjust begging intensity to intra-colony relatedness. When interpreting results obtained from comparisons between species, we additionally relied on more robust relatedness estimates obtained from larger data sets in previous studies (Sundström et al., 2003, Schultner et al., 2014, 2016).

Worker Response to Larval Odors

On average, 1.3 (± 1.3 SD) *F. fusca* workers, 0.8 (± 0.9 SD) *F. exsecta* workers, 1.3 (± 1.4 SD) *F. pressilabris* workers, and 1.2 (± 1.3 SD) *F. aquilonia* workers approached larval odors during the observation period. Workers did not move significantly more toward the odor of starved larvae than toward the odor of non-starved larvae, neither when combining data from all species nor when analyzing each species separately (**Figure 5**, **Table 4**, all species: $p = 0.61$, *F. fusca*: $p = 0.32$, *F. exsecta*: $p = 0.85$, *F. pressilabris*: $p = 0.06$, *F. aquilonia*: $p = 0.61$).

Characterization of the Larval Chemical Profiles

Overall, we found 46 peaks after exclusion of Petri dish contaminations (**Supplement Table 1**). When analyzing all species together, visual inspection of PCAs carried out with all compounds showed no difference in the cuticular hydrocarbons of starved and non-starved larvae (**Figure 6A**). Similarly, there was no difference in the cuticular hydrocarbons of starved and non-starved larvae across all species when compound groups where analyzed separately: alkanes (**Figure 6B**), methylated alkanes (**Figure 6C**), alkenes (**Figure 6D**). We also did not find strong differences between the cuticular hydrocarbon profiles of starved and non-starved larvae when analyzing each species separately (**Supplement Figures 1–4**), with the exception of *F. exsecta*. However, the apparent difference in the chemical compounds of starved and non-starved *F. exsecta* larvae may be due to the smaller number of colony replicates for this species. While we did not find any treatment effects on larval odors, the analysis did reveal differences between species (**Figure 7**). Species differences in larval odor profiles were most apparent when analyses included all compounds (**Figure 7A**) and only alkenes (**Figure 7D**). Additionally, the hydrocarbon profiles of *F. fusca* larvae differed from those of all other species in the composition of methyl-branched alkanes (**Figure 7C**).

TABLE 4 | Results of Generalized Linear Mixed effects Models linking the maximum number of workers on a glass slide to the odor of starved or non-starved larvae on this slide.

Maximum number of workers on a glass slide	**~State*species + (1|group)**			
All species ($n = 423$)	**Effect**	**Chisq**	**df**	***p*-value**
	State	0.08	1	0.781
	Species	33.94	3	**<0.001**
	State:species	4.63	3	0.201

Maximum number of workers on a glass slide	**~State + (1|group)**			
	Effect	**β ± SE**	***z***	***p*-value**
F. fusca ($n = 135$)	State	0.08 ± 0.08	0.99	0.322
F. exsecta ($n = 72$)	State	−0.02 ± 0.13	−0.19	0.847
F. pressilabris ($n = 108$)	State	−0.16 ± 0.08	−1.85	0.064
F. aquilonia ($n = 108$)	State	0.05 ± 0.09	0.51	0.613

The first model is a global model for all the species combined whereas the following models are species-specific. Significant p-values are written in bold. n represents the number of data points used in each model.

DISCUSSION

So far, there have been few studies on the influence of kinship on signaling honesty in insects. Caro et al. (2016) found that offspring from around 60 species of birds are generally less honest when facing competition from distantly related nestmates. Begging honesty has mostly been studied in birds and mammals, even though the large natural range of relatedness variation makes social insects great models for understanding the evolution of offspring signals. The results we obtained by comparing larval begging in ant species with varying kin structure are generally consistent with the idea that dishonesty is more prevalent under low relatedness, when inclusive fitness costs suffered through depriving nestmates of food are low. Larvae from high and intermediate relatedness species (*F. fusca, F. exsecta*) begged when hungry, and thus honestly signaled their need. In contrast, larvae from low-relatedness species (*F. aquilonia*), showed behaviors more resembling scramble competition or dishonest signaling. However, given the small number of species studied, and potential confounding factors that could explain the species differences discussed below, further studies are necessary for strong conclusions.

Begging intensity varied with species-specific traits in complex ways. Most importantly, we did not find an overall significant effect of intra-colony relatedness on begging levels. However, this does not mean that species-specific kin structures do not influence begging. Instead, in the global analyses we may not have been able to detect species-level changes in begging behavior caused by intra-colony relatedness variation due to the large variation in our relatedness estimates, which do not perfectly reflect estimates obtained in previous studies (e.g., Sundström et al., 2003; Schultner et al., 2014, 2016). There are two main reasons that could explain why our estimates deviate from those of previous studies. First, in order to obtain enough larvae for experiments, we preferentially used multiple queen colonies for *F. fusca,* even though these have lower relatedness levels than single queen colonies (multiple queen colonies: $r = 0.2 \pm 0.18$ SD, single queen colonies: $r = 0.68 \pm 0.12$, Helanterä et al., 2011). This likely biased intra-colony relatedness toward lower values in this species. Second, we were limited by small sample sizes, which generally results in imprecise allele frequency estimates both in group level allele frequencies and the population wide background allele frequencies, thus increasing the uncertainty of relatedness estimates (Queller and Goodnight, 1989). Nevertheless, the difference in kin structure is one of the most prominent traits that characterizes our focal species, as other traits such as nesting behavior show large overlap between the species. For example, *F. exsecta*, *F. pressilabris*, and *F. aquilonia* can found their colonies dependently in nests of other ant species, while *F. fusca* often acts as a host (Seifert, 2018). In terms of colony sizes, *F. fusca* has the smallest colonies, with *F. exsecta* and *F. pressilabris* having intermediate and *F. aquilonia* by far the largest colonies. Finally, adult size is largest in *F. aquilonia* compared to the other three species, but large intraspecific variation both within and between colonies occurs in all species.

Two measures of begging intensity were significantly affected by the interaction between state and intra-colony relatedness in the global analyses, indicating that kin structure may modulate state-dependent begging behaviors. In addition, in the species-specific analyses, increasing relatedness generally had a negative influence on begging intensity, as expected if begging were generally correlated with kin competition. Although these effects were not always statistically significant, and suffer from the uncertainty of our relatedness estimates, this suggests that kin structure may shape begging behavior both over evolutionary time (i.e., on a species level) and over shorter time spans (i.e., on a colony level). This is surprising, as begging behavior was quantified in sibling groups, and larvae presumably had no access to information about average colony kin structure. This suggests that the relatedness effect is either mediated through a maternal effect—as the mother and the workers who tend to her do have access to information on kin structure (see e.g., Helanterä and d'Ettorre, 2015)—or factors that covary with both kin structure and brood provisioning levels. Further studies that include more species with varying kin structures and, ideally, studies that experimentally manipulate relatedness and its potential covariates such as colony size, are now needed to better comprehend the impact of kin structure on larval behavior within and between species.

Within-colony sex ratios also explained some of the variation in begging intensity (but note that the same proviso about confounding variables applies here as well). Specifically, the number and duration of swaying events was higher when larval sex ratios were male-biased in *F. aquilonia*. Similar differences in begging intensity between males and females have been recorded in zebra finches (von Engelhardt et al., 2006). In addition, in *F. aquilonia* as well as over all species combined, only non-starved

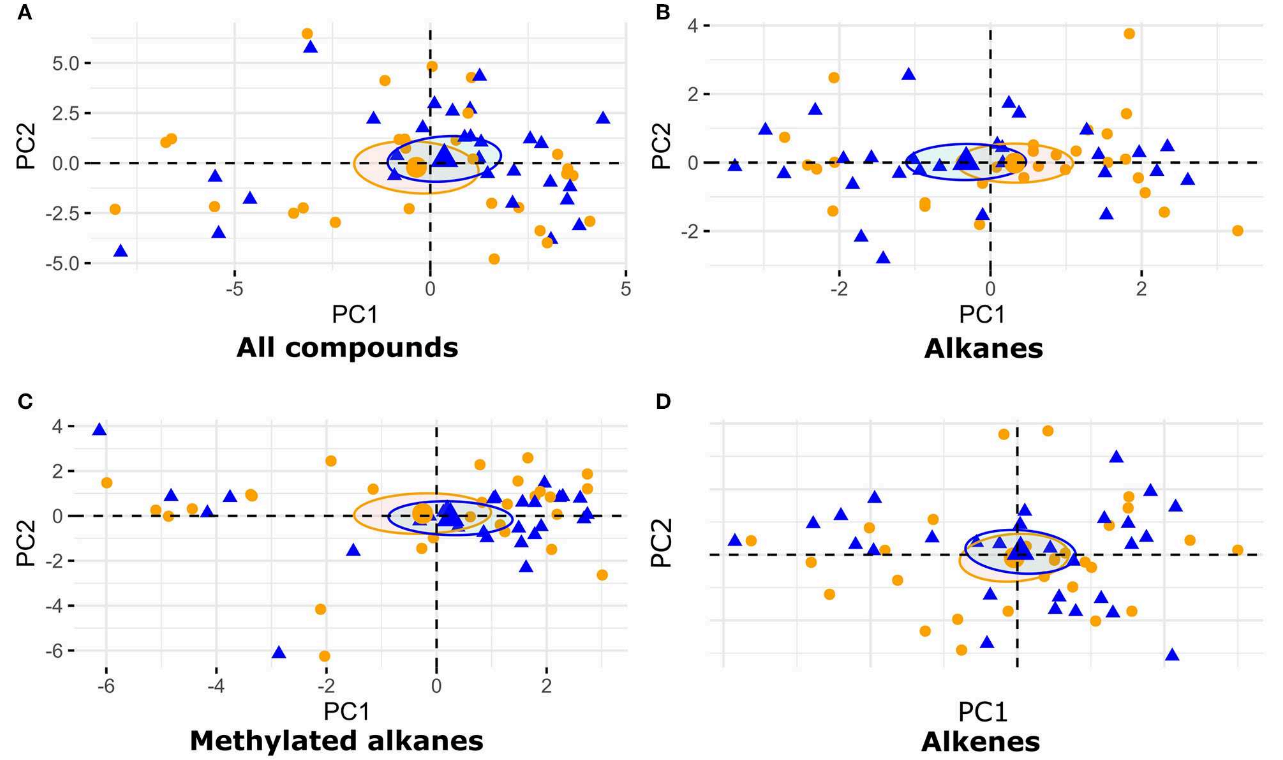

FIGURE 6 | Representation of PCAs of **(A)** all cuticular hydrocarbons, **(B)** alkanes, **(C)** methylated alkanes, and **(D)** alkenes calculated from relative proportions of cuticular compounds extracted from larvae of four *Formica* species. Non-starved larvae are represented in orange and starved larvae are represented in blue. The circles represent the 95% confidence ellipse for each group.

larvae swayed more often in response to male-biased sex ratios. Selfishness has been shown to be more common in male *Formica* larvae in the context of cannibalism (Schultner et al., 2013, 2014), and males may be under selection to compete more fiercely for resources, in particular if nutritional gains increase fitness-related traits such as sperm quantity, quality (Delisle and Hardy, 1997) or adult body size (Wiernasz et al., 1995; Osawa, 2002). In addition, if competition for mating opportunities among males is stronger than among females, male-biased sex ratios may result in high levels of male-male competition. Such competition may be especially intense when larvae can afford to expend energy for begging, i.e., when they are in a non-starved state. In ants, selfish larval behavior in the form of cannibalism is predicted to depend on an interaction between kinship and sex ratio (Schultner et al., 2014), and the complex patterns observed here suggest that similar models, which take into account haplodiploidy and kin structure variation, are needed for a better understanding of begging in social insects.

One factor that may potentially confound the results is the age of larvae. Indeed, it is possible that older, starved, larvae are simply more experienced and better at begging than young, non-starved larvae. Conversely, it is possible that young, non-starved, larvae had more resources to invest in begging, resulting in more intense begging. Finally, contrasting patterns of begging intensity in the focal species may stem from differences in larval size, developmental rates or metabolism, rather than from species-specific responses to nutritional status. While these are plausible alternative explanations, there are several reasons why we think begging intensity is likely more dependent on nutritional status than larval age. First, the behavior of starved and non-starved larvae in *F. pressilabris* was very similar, indicating that larvae know how to beg innately and can do so regardless of their age or nutritional status. Second, in *F. exsecta* and *F. fusca* older, starved larvae begged more than young, non-starved larvae, indicating that starved larvae were not too deficient in energy to prevent them from begging. Finally, like in other insects, temperature is an important factor influencing development rates in ants (e.g., Porter, 1988). As larval rearing and behavioral assays were conducted under controlled temperature conditions, it is unlikely that larvae from different species underwent development at fundamentally different rates.

While we found clear patterns when looking at the number of swaying events, the patterns of swaying duration, and the number and duration of mandible movement, were not as clear; nevertheless, the data revealed similar trends. In *Gnamptogenys striatula* ants, Kaptein et al. (2005) found that starved larvae swayed significantly longer than non-starved larvae in the presence of workers. It is thus possible that swaying duration depends on the presence of workers, which reflects the difficulty of interpreting behaviors of isolated larvae. It is also possible

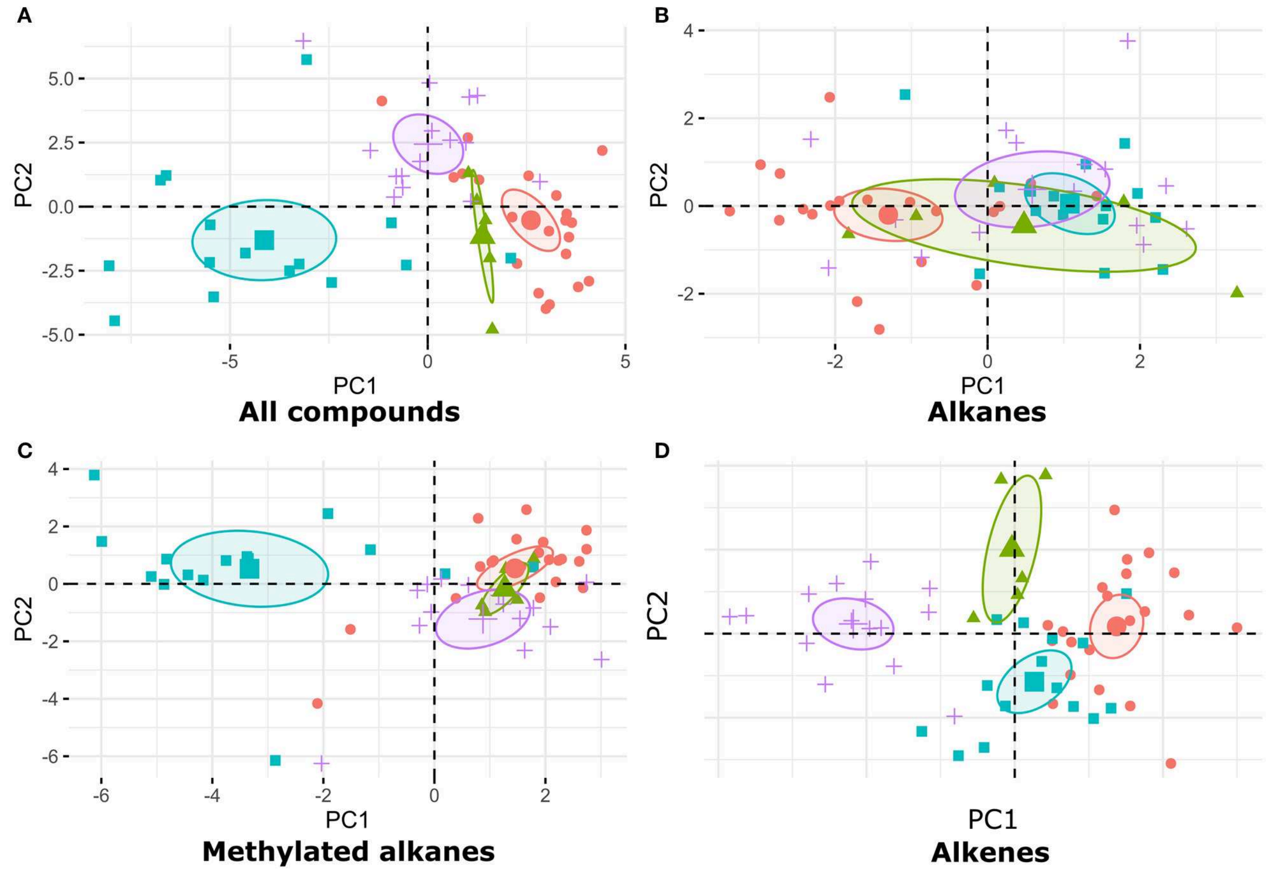

FIGURE 7 | Representation of PCAs of **(A)** all cuticular hydrocarbons, **(B)** alkanes, **(C)** methylated alkanes, and **(D)** alkenes calculated from relative proportions of cuticular compounds extracted from larvae. *F. fusca* larvae are represented in blue, *F. exsecta* in green, *F. pressilabris* in violet, and *F. aquilonia* in red. The circles represent the 95% confidence ellipse for each group.

that mandible movements are not related to begging, or that the quality of the videos did not allow us to correctly count all incidents of mandible movement.

As signaling through cuticular hydrocarbons in social insects is particularly well-developed (e.g., Blomquist and Bagnères, 2010), we hypothesized that larvae emit chemical hunger signals, and that workers should preferentially respond to odors of starved larvae. This hypothesis was based on previous studies showing that *Bombus terrestris* workers reacted to extracts of starved larvae with increased feeding (den Boer and Duchateau, 2006), and that female burrower bugs provisioned more when exposed to extracts from nymphs reared in low food conditions (Kölliker et al., 2005). In contrast to these results, *Formica* ant workers did not preferentially approach the odors of starved larvae compared to the odors of non-starved larvae. In line with this, chemical analysis of larval cuticular hydrocarbon profiles did not reveal any differences between starved and non-starved larvae. Nevertheless, there are several reasons why we cannot rule out that chemical hunger signaling plays a role in *Formica* ants. First, we may have overlooked potential chemical signals, for instance because relevant compounds are more polar, their concentrations lay below the detection limit, or because the compounds had lower or higher molecular weights than those included in our analysis. Second, our study was not designed to test for volatile compounds such as those known to play a role in larval chemical hunger signaling in honey bees (He et al., 2016). Third, chemical signaling by larvae may be context-dependent, and, if it is costly, may only occur in the presence of workers. Finally, workers may respond to larval chemical signals only when they are accompanied by other visual, tactile or behavioral cues. In the ant *Formica cunicularia* for example, workers accept heterospecific brood only when it resembles conspecific brood in both size and odor (Mori and Le Moli, 1988). Further studies are thus needed to rule out the existence of chemical hunger signals in *Formica* larvae.

Although we did not find any hunger-related differences in larval cuticular chemistry, chemical analyses confirmed the presence of species-typical chemical profiles (Martin and Drijfhout, 2009) in *Formica* ant larvae. However, some inconsistencies remain. For instance, not all *F. fusca* samples clustered together. This suggests that larval odors are less strong, or less species-specific, than adult odors (Carlin, 1988), or that individual colonies have very clear and distinct profiles. The last hypothesis is particularly likely for high-relatedness species, where each colony is genetically distinct. Indeed, the chemical profiles of *F. fusca* eggs are known to be more colony-specific than those of species with lower relatedness such as *F. aquilonia* (Helanterä and d'Ettorre, 2015). Chemical analyses of larval odors also highlighted an interesting phenomenon. Previous studies on cuticular hydrocarbons of ants have typically focused on eggs and adults (van Zweden and d'Ettorre, 2010). In some of these studies, individuals were placed on Petri dishes before being moved to glass vials for extraction. Our samples were handled in a similar manner, and we consistently observed contamination of larval profiles, most likely originating from Petri dishes. This may be due to the fact that, unlike eggs, which are protected by a chorion, freshly hatched larvae like those used in our experiments lack thick cuticles. In natural colonies, this may allow young larvae to pick up colony odors easily. As the only available studies of larval cuticular chemistry in ants have focused on larvae in later stages of development (Brian, 1975; Hare, 1996; Akino et al., 1999; Viana et al., 2001; Villalta et al., 2016; Penick and Liebig, 2017), further studies are required to assess how development stage-dependent differences in cuticle composition may affect odor acquisition.

Begging as a classic conflict-related trait has been largely overlooked in social insects. In particular, the importance of chemical signals as indicators of nutritional state, and the impact of relatedness on the honesty of begging signals have not yet been studied. Our study revealed that, in *F. fusca* and *F. exsecta*, starved larvae begged more frequently than non-starved larvae. In these species with predominantly high intra-colony relatedness, this can be interpreted as an honest signal of need. This is in line with what has been found in *Myrmica rubra* (Creemers et al., 2003) and *Gnamptogenys striatula* (Kaptein et al., 2005). Accordingly, *G. striatula* nests exhibit relatively high levels of relatedness ($r = 0.65 \pm 0.25$ SE, Giraud et al., 2001). In *M. rubra*, intra-colony relatedness varies strongly depending on the study population ($r = 0$–0.82, Seppä and Walin, 1996). As relatedness among *M. rubra* larvae was not estimated in the previous study, it is difficult to draw conclusions about the influence of kin structure on begging honesty in this species. In contrast, larvae in low-relatedness species were more inclined to convey dishonest signals about their state. Thus, as predicted by inclusive fitness theory, begging in social insects may be influenced by colony kin structure. However, it remains unclear whether dishonestly signaling larvae from low-relatedness nests actually receive more food from workers as a result of increased begging, i.e., whether the dishonest signal pays off. Furthermore, our study does not allow us to infer how individual traits such as future reproductive caste may influence a larva's propensity to beg. For example, individual begging intensities may vary with species-level traits such as the degree of queen-worker dimorphism, with more begging expected to occur when the dimorphism is more pronounced. As caste of developing females has previously been shown to affect worker behavior (e.g., Brian, 1973; Passera et al., 1995; Penick and Liebig, 2012, 2017), it is furthermore possible that larval caste affects worker responses to begging.

Overall, this study provides the first test of inclusive fitness predictions in the context of hunger signaling in social insects. By demonstrating that the honesty of larval hunger signaling varies among species with different colony kin structures, our study highlights yet another social conflict in which kinship covaries with social insect behavior, thus providing further evidence that larvae are active players in colony life (Schultner et al., 2017).

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

MP, HH, and ES contributed conception and design of the study, and carried out field collections. MP, RL, TP, and ES collected the data. MP, TP, ES, and HH analyzed the data. MP wrote the first draft of the manuscript. MP, TP, JH, HH, and ES contributed to manuscript revision and all authors read and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00398/full#supplementary-material

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Sight in a Clique, Scent in Society: Plasticity in the Use of Nestmate Recognition Cues Along Colony Development in the Social Wasp *Polistes dominula*

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Nestmate recognition, i.e., the ability to discriminate nestmates from foreign individuals, is a crucial feature of insect societies, and it has been traditionally considered to be predominantly based on chemical cues. Recent empirical evidence, however, suggests a relevant plasticity in the use of different communication channels according to cue availability and reliability in different contexts. In particular, visual cues have been shown to influence various types of social recognition in several social insects, but their role in nestmate recognition is still under-investigated. We tested the hypothesis of plasticity in the use of visual and chemical recognition cues in the primitively eusocial wasp *Polistes dominula*, in which the availability and reliability of recognition cues vary across the colony cycle. Indeed, before the emergence of workers, *P. dominula* colonies are rather small (one to few individuals), and the variability in the facial pattern might allow resident wasps to use visual cues for nestmate recognition. After workers' emergence, the increase in the number of colony members reduces the reliability of visual cues, thus leaving chemical cues as the most reliable nestmate recognition cues. We thus predict a differential use of chemical and visual cues along colony life. We experimentally separated visual and chemical cues of nestmates and non-nestmates and presented them alone or in combination (with coherent or mismatched cues) to resident wasps to test which communication channel was used in the two stages and, in case, how visual and chemical cues interacted. Our results show, for the first time in a social insect, the differential use of visual and chemical cues for nestmate recognition in two different phases of colony, which supports the hypothesis of a plastic, reliability-based use of recognition cues in this species according to the different colonial contexts.

Keywords: cuticular hydrocarbons, multimodal communication, paper wasps, familiar recognition, phenotypic plasticity

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INTRODUCTION

Social organization relies upon social recognition, which is the ability of individuals to distinguish among the individuals they encounter and to bias their behavior accordingly, i.e., responding with an adaptive behavior toward the appropriate individual (Ward and Webster, 2016). Social recognition thus plays a crucial role in regulation of social interactions within animal groups, by shaping parent–offspring interactions, competitive aggression, mate choice, and cooperative behaviors (Waldman, 1988; Gherardi et al., 2012; Aquiloni and Tricarico, 2015). Eusocial insects, such as ants, wasps, termites, and bees, live in complex societies that represent pinnacles of social evolution and whose organization relies on sophisticated forms of social recognition, such as the ability to recognize caste, dominance and fertility status, gender, and nestmates from non-nestmates (Wilson, 1971; van Zweden and d'Ettorre, 2010; Cervo et al., 2015).

Nestmate recognition (hereafter NMR), i.e., the ability to discriminate nestmates from non-nestmates, is the quintessential form of social recognition that occurs in insect societies (d'Ettorre and Lenoir, 2009). Social insect colonies are rich in resources that conspecific and heterospecific individuals may exploit: nests are costly to produce and advantageous in the protection they provide, colonies are full of harmless and meaty brood, and workers efficiently provide alloparental care that might be selfishly exploited. Many species across the whole range of the animal kingdom indeed benefit from exploiting social insect colonies at various extents, from predation to social parasitism (Fürst et al., 2011; Cini et al., 2019). NMR evolved to allow colony members to recognize and accept each other while strongly repelling potentially dangerous intruders, thus allowing the protection of the colony and directing altruistic acts toward related recipients (Hamilton, 1987).

NMR occurs through a process of phenotype matching that involves the perception of a label carried by encountered individual and the comparison of this label with an internal reference (template), i.e., a neural representation of the trait stored within the evaluator peripheral and central nervous system (Crozier and Pamilo, 1996; Leonhardt et al., 2007; d'Ettorre and Lenoir, 2009; Signorotti et al., 2015). The response of the evaluator depends on how well the label matches the template (van Zweden and d'Ettorre, 2010), with the aggressive response triggered when the mismatch exceeds a certain threshold (Reeve, 1989). Decades of research convincingly demonstrated that colony identity is mainly encoded in the blend of cuticular hydrocarbons (CHCs) (Howard and Blomquist, 2005; Blomquist and Bagnères, 2010). Typically, colonies of a given species have a qualitatively similar CHC profile, which differs in the relative amounts of each compound (Lorenzi et al., 1996; Dani, 2006; Bruschini et al., 2010; van Zweden and d'Ettorre, 2010).

CHC blends have several advantages as NMR cues compared to other potential cues pertaining to different sensory modalities. First, the CHC blend usually entails several dozens of compounds, which vary in their relative abundance across colonies, so that the signal arising from such a complex mixture can be informative about colony membership (van Zweden and d'Ettorre, 2010; Sturgis and Gordon, 2012). Then, CHC blend profile is highly influenced by the environment (e.g., by diet, Liang and Silverman, 2000; Buczkowski et al., 2005) and CHCs can be exchanged through social contact, which makes the CHC signal highly flexible, thus enabling to keep the colony signature updated in a continuously changing environment (Richard and Hunt, 2013).

While it has been repeatedly shown that CHCs are the main cues used in NMR (reviewed in Blomquist and Bagnères, 2010; van Zweden and d'Ettorre, 2010), recent experimental evidence revealed that olfaction might be coupled with, or even overcame by, other sensory modalities, such as vision (Cervo et al., 2015). Indeed, in a tropical hover wasp species characterized by small and flexible societies, *Parischnogaster flavolineata*, colony members are able to perform NMR using individual facial patterns in addition to chemical cues, and in case of contrasting information, visual cues are preferred over chemical ones (Baracchi et al., 2013, 2015).

The importance of visual cues in social insect recognition remained overlooked for many decades. The last 15 years of researches, especially in paper and stenogastrine wasps, provided strong empirical evidence about the use of visual cues in several forms of social recognition inside and outside social insect colonies, both in the intraspecific and interspecific context (Cervo et al., 2015). Wasps do indeed show remarkable variation in the color patterning of faces and abdomen and the use of such cues in social recognition has been shown for almost all species (even if few) investigated so far. This suggests that this ability could be widespread in social wasps, especially in those that live in nests without envelopes, where communication by using reflected light to produce visual signals is possible (reviewed in Cervo et al., 2015).

Despite the potentially smaller informative content of visual cues compared to chemical ones (but see Baracchi et al., 2016) and their static nature (individual color patterning remains stable after emergence, while CHC blend is continuously updated), visual cues might be advantageous over chemical ones to enable NMR as they can be quickly processed and do not require contact or really close distance (contrary to CHCs), thus enabling a faster NMR decision. Indeed, when assessing a potential intruder, colony members are faced with a trade-off between speed and accuracy of recognition and, depending on the context, speed might be prioritized over accuracy (Chittka et al., 2009; Baracchi et al., 2015). We can thus predict visual cues to be mostly used in species characterized by small colonies, where repeated encounters with a low number of colony members might allow learning their visual pattern through a familiarization process. Given that small societies are indeed common in many social insect groups, the use of visual cues in NMR potentially involves many species, especially in the primitively eusocial taxa that represent an interesting experimental window on the evolution of sociality (Rehan and Toth, 2015).

Our current understanding of the cues underlying NMR in insect societies thus suggests an association between the sensory channel used for NMR and colony size. Chemicals might be preponderant in large societies, such as in honeybees and many ant species, where visual cues could not clearly be reliable, while visual cues might be involved (together with or replacing chemicals) in small societies with variable visual cues (such as those of paper wasps). In some insect species, however, such as independent founding wasps, colony size dramatically changes throughout the colonial development, passing from few to hundreds of colony members (Reeve, 1991). A compelling question, so far unanswered, is therefore to what extent a species can plastically shift from using cues of one sensory modality (e.g., visual) to those of another one (e.g., chemical) during the colony development. In other words, we wonder if the sensory modality used for NMR is hardwired within the species behavioral repertoire or can change according to the availability and reliability that it assumes in different colonial phases. By answering this question, we aim to unveil an unexpected and yet undocumented level of plasticity in insect communication and to provide an experimental model system for future studies regarding cognitive abilities of social insect mini-brains.

Here, we tested the hypothesis of plasticity in the use of visual and chemical recognition cues according to their reliability as NMR cues in the primitively eusocial wasp *Polistes dominula*, i.e., that NMR is based on different cues in different phases of the colony cycle (**Figure 1**).

P. dominula is a temperate paper wasp species whose small colony size and phenotypic plasticity have made it a model organism for social evolution and communication studies (Pardi, 1948, 1996; Dani, 2006; Jandt et al., 2014; Cervo et al., 2015) and, thanks to the recent release of its sequenced genome, also for *omic* studies (Standage et al., 2016). *P. dominula* species also represents a good model to test the existence of plasticity in the use of visual and chemical cues according to their availability and reliability, as (i) both chemical and visual cues are known to be used in several forms of social recognition, and (ii) availability and reliability of recognition cues vary across the season (Dani, 2006; Cervo et al., 2015) (**Figure 1A**).

NMR in *P. dominula* is behaviorally evident, with non-nestmates that are highly repelled through aggressive reactions by resident wasps (Dani et al., 2001) and it is based on chemical cues, in particular in the variation in CHC signature among different colonies (Bruschini et al., 2011). On the contrary, facial color patterns, which are widely variable in this species and consists of one or more black spots, with variable size and shape, or no black spots at all on the yellow clypeus, are used for different social recognition forms, such as signaling of dominance status and agonistic abilities (e.g., Tibbetts and Dale, 2004; Tibbetts and Lindsay, 2008, but see Cervo et al., 2008), gender recognition (Cappa et al., 2016) and possibly species recognition (Cervo et al., 2015; Cini et al., 2015), but they have never been shown to allow NMR (Cervo et al., 2015).

Here, we experimentally separated visual and chemical cues of nestmate and non-nestmate *P. dominula* wasps and presented the cues alone or in combination (with coherent or mismatched cues) to resident wasps in NMR behavioral trials (**Figure 1B**). We aimed to test which communication channel is used in the two different stages of the colony cycle (at the beginning, when colonies are inhabited by only a few individuals and, after the emergence of workers, when the number of colony members dramatically increases, **Figure 1A**) and, if so, how visual and chemical cues interacted. Our prediction was that a differential use of chemical and visual cues occurs along colony life, with visual cues used only, if ever, in the pre-emergence period, while chemical cues would be used in both periods (**Figure 1B**). To our knowledge, our results show, for the first time in a social insect, a differential use of chemical and visual cues across the colony cycle, and provide the first experimental proof that, in this species, visual cues, in addition to chemical cues, are used to recognize nestmates from non-nestmates.

MATERIALS AND METHODS

Animal Collection and Laboratory Rearing

P. dominula colonies are founded in early spring, when one or more females build a new colony and take care of the immature brood (pre-emergence phase). At the end of May, the first brood emerges: these females are workers that do not reproduce but rather take care of the nest and of the immature brood (post-emergence phase). Reproductive individuals, males and gynes, emerge only later in the season, from the end of July (reproductive phase) (Reeve, 1991). Mating occurs outside of the colony at the end of summer (Beani, 1996); mated females overwinter in large groups and then start new colonies in the following spring (Dapporto and Palagi, 2006; Cini and Dapporto, 2009).

For the first experiment (pre-emergence phase), 36 bigynic colonies (i.e., colonies founded by two foundresses) were collected, during the first half of May 2015, before worker emergence, from three different sites throughout Tuscany (Central Italy). In the same period, foundresses from a different population were collected to be used as non-nestmate lures (see below).

For the second experiment (post-emergence phase), 10 colonies in workers' phase (with at least 5 workers) were collected in the same sites at the beginning of July 2016. Non-nestmate workers used as lures were collected on colonies belonging to different populations located in the same area (Tuscany, Central Italy). In both experiments the wasps used as non-nestmates were collected in populations at least 3 km apart from the populations where focal experimental colonies were collected, in order to minimize the likelihood of high relatedness and prior encounter among tested individuals.

Colonies were brought to the laboratory, and each colony was transferred to a 15 cm × 15 cm × 15 cm glass cage provided with *ad libitum* sugar, water, fly maggots, and paper as nest-building material. Colonies were maintained under natural light cycle and temperature conditions with additional illumination from neon lighting with a daily rhythm (L:D 10:14).

In the bigynic nests, each foundress was individually marked with a different combination of enamel colors (Humbrol, UK) on the wings for individual identification. Behavioral observations were carried out before NMR experiments in order to establish the dominant individual for each colony, on the basis of well-established dominant rank-related behaviors such as ritualized dominance behaviors, egg-laying, and low foraging effort (Pardi, 1948; Pratte, 1989).

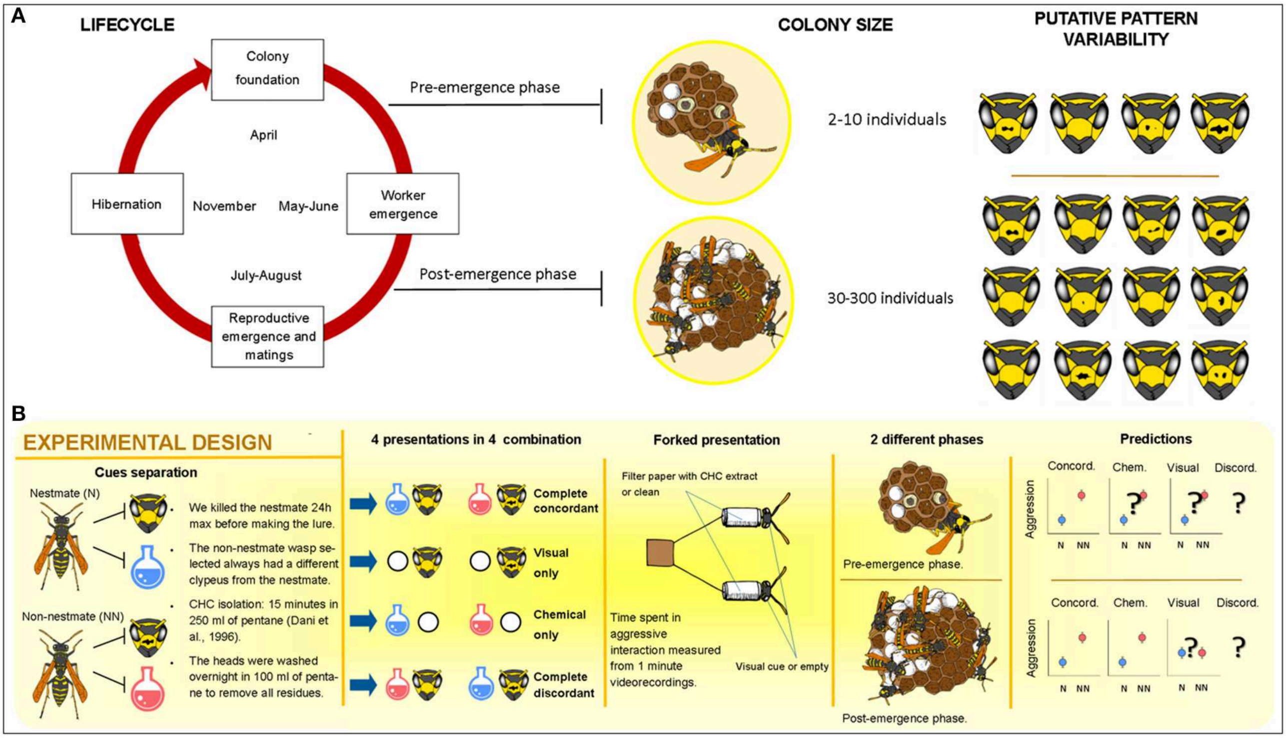

FIGURE 1 | (A) Life cycle of *Polistes dominula*: the two phases considered in this work are shown together with their variation in colony size and the putative facial pattern variability; **(B)** the experimental design used in the study. Our prediction was that a differential use of chemical and visual cues occurs along colony life. When both stimuli (chemical and visual) are presented together (concordant lure), proper nestmate recognition is expected [i.e., Non-nestmates (NN) are attacked more than Nestmates (N)]. When stimuli are presented alone (i.e., only chemical or only visual), the presence of proper recognition will depend on the reliability of cues. We predict that in the pre-emergence stage, both single-cue lure (visual and chemical) will elicit proper NMR. On the contrary, we predict that in the post-emergence stage, proper NMR only occurs when chemicals are presented, while no NMR will occur when only visual cues are presented. No a priori prediction about discordant lures (conflict) can be made without knowing the results of single-cue lures. Question marks indicates that no previous experimental evidence has ever been produced for such a comparison (Drawing: Leonardo Platania).

General Experimental Procedure

Each colony was subjected to four NMR trials, which consisted in the simultaneous presentation of two lures carrying NMR cues related to one or both sensory modalities—visual (i.e., an odorless wasp head) and chemical (i.e., CHCs) cues—in a concordant (both from the same individual, which could be a nestmate or a non-nestmate) or discordant (one from a nestmate and the other from a non-nestmate individual) combination (**Figure 1B**). Lures were presented to colonies in a random order and behavioral response was video-recorded for 1 min after the first interaction between lure and resident wasps. An aggressive response index was computed as the total number of aggressive acts (bites and stings) performed toward the lure (see *Data Analysis* below). The aggressive response of the colony as a whole was measured. This corresponds to the aggressive reaction of the alpha female, the only wasp present in the colony, in the pre-emergence phase, and to the aggressive reaction of all the workers that responded in the post-emergence phase. Both pre-emergence and post-emergence colonies were tested once with the same protocol.

Lure Selection

In the pre-emergence experiment, for each colony, two wasp lures were selected: (i) the beta female of the tested nest as nestmate lure and (ii) a foundress belonging to a different population as non-nestmate lure. Lures were coupled based on a clear different color pattern on the clypeus, i.e., the non-nestmate lure was chosen randomly within a pool of wasps with a clypeus patterning different from that of the nestmate wasp. Three categories of clypeus pattern were selected: 1 = totally yellow clypeus, 2 = one spot, and 3 = two or more spots on the clypeus (Tibbetts and Lindsay, 2008).

Analogously, also in the post-emergence one, two wasp lures with a different color pattern on clypeus (see above) were selected for each colony: (i) a worker of the tested nest as nestmate lure and (ii) a worker belonging to a different population as non-nestmate lure. The frequency distribution of clypeus pattern was not different between treatments in either experiment ($\chi^2 = 2.19$, df = 2, $p = 0.335$; $\chi^2 = 5.01$, df = 2, $p = 0.082$). Apart from the clypeus pattern, all lure wasps were randomly chosen. Lure size (estimated by measuring head width, Cini et al., 2011a) was not different among treatments or in the pre-emergence experiment (Wilcoxon test, W: 369 $p = 0.802$, $n = 37$) or in the post-emergence one (Wilcoxon test, W: 369 $p = 0.349$, $n = 20$).

We used alpha females as resident focal females (and thus we used the removed beta females as nestmate lures) for the pre-emergence phase experiment for both biological and experimental reasons. From the biological point of view, the reaction of alpha females is expected to be more uniform than that of beta females. Indeed, while the alpha female must defend her nest against any kind of individual, the beta female might have divergent interests according to the identity and strength of the opponent. While beta females usually show colony defense and NMR, in some cases, they might accept (or attack to a different degree) a very dominant individual. This might occur as beta females are defending a resource (the colony and the brood therein) that represents a smaller fitness gain to them than to alpha females (only the alpha females reproduce and alpha females and beta females are often unrelated, Queller et al., 2000). From the experimental point of view, in order to do the test and present a nestmate together with a non-nestmate as lures, we needed to kill the nestmate (the beta female in our case). If it was the alpha female to be killed and used as a lure, we would have created an orphan colony, even if for just a few hours. This would have been different from the post-emergence phase, where we would have removed a worker (to be matched with a non-nestmate worker), so we would have another (and bigger) difference.

Lure Preparation

Chemical Cues

All wasps selected to be used as a lure were killed by freezing 1 day before the bioassay. For obtaining CHC extract, the entire body of each wasp lure was individually placed in a glass vial with 250 µl of an apolar solvent (pentane) for 15 min (Dani et al., 1996). After wasp body removal, vials with pentane extracts were left to dry overnight. The following day, before NMR bioassays, extracts were resuspended in 100 µl of pentane and transferred on pentane-washed filter paper sheets (2.7 × 1 cm). Filter paper sheets were then fixed on an inert support (half filter tip, ultraslim, Rizla) to obtain lures bearing the sole chemical cues (**Figure 1B**).

Visual Cues

After washing the wasp body in pentane, the head of each lure was separated from the rest of the body and kept in 1 ml of pentane overnight (Cini et al., 2015) to totally remove the residual CHC fraction. The following day, before NMR bioassays, the heads were mounted on entomological pins over the inert support (see above).

Lure Presentation

During each of the four NMR experiments, two lures were simultaneously presented to each colony (following a procedure already tested for both visual and chemical stimuli; Ortolani et al., 2010; Bruschini et al., 2011; Cini et al., 2011b, 2015). Each lure was composed of one out of four possible combinations of stimuli obtained by nestmate and non-nestmate wasps (**Figure 1B**): (i) "only visual" lures, i.e., odorless heads of nestmate (see above) and non-nestmate wasps mounted on pentane washed paper filters; (ii) "only chemical" lures, i.e., filter tip with filter paper sheet loaded with CHC extracts of nestmate and non-nestmate; (iii) concordant lures, bearing together chemical and visual cues of each wasp, recreating the natural coupling of visual and chemical cues where each individual presents its own array of stimuli; and (iv) discordant lures, composed of nestmate visual cues (head) and non-nestmate chemical cues (scent) on one lure and non-nestmate visual cues and nestmate chemical cues on the other, creating an artificial combination of mixed visual and chemical stimuli. In both pre-emergence and post-emergence experiments in the discordant treatment, the lure with the visual stimulus of the non-nestmate (and thus the chemical stimulus of the nestmate) was considered as the "non-nestmate" lure and the lure with the visual stimulus of the nestmate (and thus the chemical stimulus of the non-nestmate) was considered as the "nestmate" lure (**Figure 1B**).

The procedure for all the experiments consisted of the simultaneous presentation of two stimulus lures. Following a protocol reported for similar bioassays carried out on the same species (Ortolani et al., 2010; Bruschini et al., 2011; Cini et al., 2015), we used a 30 cm-long stick with a fork at one end. The two different lures (belonging to same combination of stimuli) were mounted on the tips of the fork, 1.5 cm apart, and were randomly placed on the left or right. The fork device was slowly introduced into the colony box while the alpha female (pre-emergence experiment) or the workers (post-emergence experiment) were on the nest, and held at a distance of 1 cm from the comb for 1 min after the first interaction between the alpha female/workers and the presented lures. In the post-emergence experiment, for each of 10 colonies, we performed two set of tests, each one presenting the lures to a group of different workers (i.e., in total, we tested 20 groups of 5–10 wasps in the whole post-emergence experiment). As in post-emergence colonies, the queen only rarely participates in NMR, and we temporary removed it from the colony for the duration of the test. Colonies were presented with all four combinations of stimuli in a random order at 1 h interval between successive trials (a trial is the simultaneous presentation of the two lures). Presentations were video-recorded. Experiments were carried out from 11:00 AM to 3:00 PM, on sunny days. All experiments were performed blindly by a first experimenter and video-recorded by a second experimenter. A total of 144 trials on 36 colonies were carried out in the first experiment (pre-emergence phase) and 80 trials on 20 different groups of workers from 10 colonies in the second experiment (post-emergence phase).

Data Analysis

Video recordings were watched blindly by two observers to avoid biases in counting the interactions between the wasps and the lures during presentations. We measured the number of aggressive acts performed (i.e., wasps open their mandibles and attack the presented lure biting and, more rarely, stinging the lure), as this is the typical behavioral response that allows the evaluation of NMR (Dani et al., 2001) toward each of the two presented lures.

In order to evaluate NMR, we assessed whether, as expected, aggressive response was significantly greater toward non-nestmate lures than toward nestmate ones, and whether this depended on the kind of stimuli presented, i.e., visual, chemical, and their combination (see above, **Figure 1B**). For each experiment, we separately used a generalized mixed model,

TABLE 1 | Nestmate recognition is based on visual or chemical cues according to the colonial stage.

Source of variance	*F*-value	df1	df2	Sig.	*F*-value	df1	df2	Sig.
		Pre-emergence				Post-emergence		
Treatment	5.099	3	292	0.002	95.119	3	152	<0.001
Lure category	67.026	1	292	<0.001	88.840	1	152	<0.001
Treatment × Lure category	9.378	3	292	<0.001	27.349	3	152	<0.001

Results from GLZ show that in both the pre-emergence and post-emergence periods, the number of aggressive acts performed toward the lure depends on the lure category (non-nestmate vs. nestmate), on the focal colony treatment (complete concordant, complete discordant, only chemicals, and only visual cues), and on the interaction between these two factors. Sig. = p-value.

with Poisson distribution and log-link function, followed by *post-hoc* pairwise comparison with sequential Sidak correction. We set the aggressive reaction (i.e., time spent in aggressive acts toward the lure) as the dependent variable. We used as predictors lure category (i.e., nestmate or non-nestmate) and treatment (i.e., only visual, only chemicals, concordant, and discordant stimuli) as fixed factors together with their interaction (lure × treatment). We considered colony id as random factor (each colony performed multiple trials). Under our predictions (**Figure 1B**), we expect to find an effect of lure (nestmate vs. non-nestmate, with non-nestmate eliciting a greater aggression than nestmate), an effect of treatment (with some cues and/or a combination of cues eliciting a greater reaction than others), and an effect of the interaction (the difference in aggression toward nestmates and non-nestmates depends on the kind of treatment, i.e., cues, presented).

This model could not include the variable "pattern of clypeus" of the lures as the lures of the "only chemical" treatment were represented by only filter papers with chemicals, and they were both bearing any information about the clypeal pattern. Thus, in order to assess the influence of clypeus pattern on the aggressive response of resident wasps, in the case that NMR was found in the visual treatment in the *post-hoc* comparisons of the first model, a second model was run. This model had the same settings of the first one, but it has been run by excluding the chemical only treatment and including the lure clypeus pattern (1 = totally yellow clypeus, 2 = one spot, 3 = two or more spots on the clypeus) together with lure category, treatment, and their interactions (lure category × treatment, lure category × lure clypeus pattern, and treatment × lure clypeus pattern).

RESULTS

In both experiments, aggressive response was significantly influenced by treatment, lure category, and their interaction (**Table 1**).

Treatment influenced aggressive response in a similar way in both experiments, with treatments involving both chemical and visual stimuli together (concordant and discordant treatments), which overall evoked more aggression, and the treatment presenting only chemical stimuli evoking the lowest levels of aggression. In particular, in the pre-emergence experiment, the highest levels of aggression were evoked by the concordant treatment, followed by discordant, only visual, and then only chemical. *Post-hoc* comparisons were significant for the only chemical vs. concordant treatment comparison ($p < 0.001$) and close to significance threshold for the only chemical vs. only visual ($p = 0.057$), for the only chemical vs. discordant ($p = 0.060$) and for the only visual vs. discordant ($p = 0.061$) treatment comparison, while non-significant for the concordant vs. discordant treatment comparisons ($p = 0.410$). In the post-emergence experiment, the highest level of aggression was found in the discordant treatment, followed by the concordant one, then the only visual, and then the only chemical treatment. All pairwise comparisons were statistically significant (concordant vs. only chemical $p < 0.001$; concordant vs. visual $p = 0.043$; concordant vs. discordant $p = 0.017$; only chemical vs. only visual $p < 0.001$; only chemical vs. discordant $p < 0.001$; only visual vs. discordant $p < 0.001$).

Lure category affected the aggressive response in a similar way in both experiments: non-nestmate lures were attacked more than nestmates (**Table 1**). A significant interaction lure category × treatment highlighted that the kind of treatment influenced the differential aggressive response toward nestmate and non-nestmate lures in both experiments (**Table 1**; **Figure 2**). *Post-hoc* comparisons showed that the kind of stimuli allowing efficient NMR was different in the two experiments. In the pre-emergence experiment, non-nestmate lures were attacked more than nestmate ones (thus highlighting a proper NMR) when complete concordant stimuli (chemical and visual) and only visual stimuli were presented ($p < 0.001$ in both cases; **Figure 2A**). On the contrary, no significant difference was found in the aggressive response toward nestmate and non-nestmate lures or when only chemical stimuli or when discordant stimuli were presented ($p = 0.174$ and $p = 0.142$, respectively; **Figure 2A**). In the post-emergence period, non-nestmate lures were attacked more than nestmate ones (thus highlighting a proper NMR) when complete concordant stimuli (chemical and visual) and only chemical stimuli were presented ($p < 0.001$ in both cases; **Figure 2B**). On the contrary, no significant difference was found in the aggressive response toward nestmate and non-nestmate lures, neither when only visual stimuli nor when discordant stimuli were presented ($p = 0.318$ and $p = 0.341$, respectively; **Figure 2B**).

The effect of clypeus pattern on aggressive response was investigated only for the pre-emergence experiment, as in the post-emergence phase, a significant NMR was not found in the visual treatment. In addition to confirming the significant effects of lure category, treatment, and their interaction, as in the first model, the main result of this second model is that clypeus

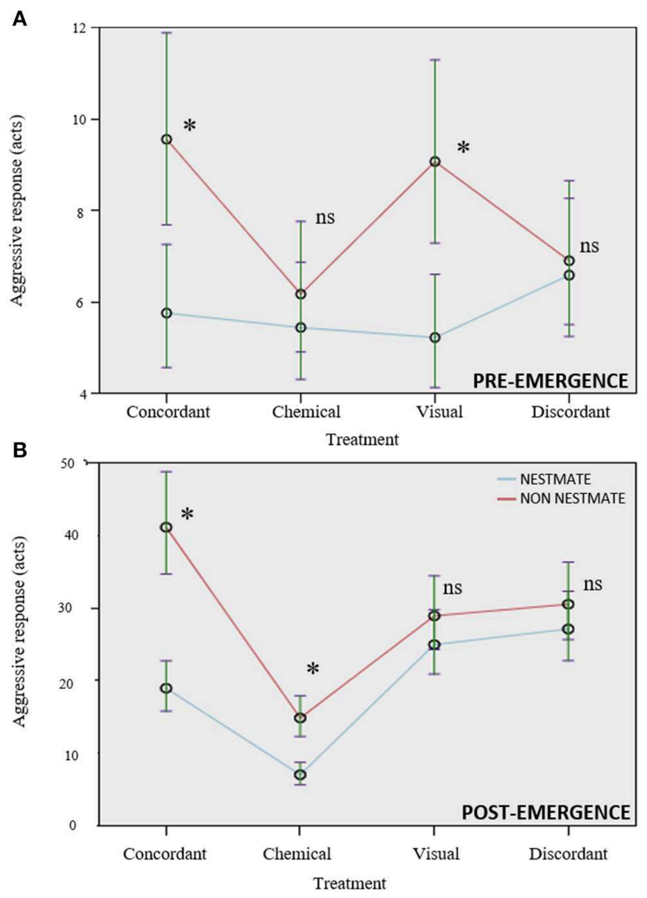

FIGURE 2 | NMR is expressed when complete concordant lures are presented, and not when complete discordant lures are presented. Visual cues alone allow NMR only in the pre-emergence period **(A)** while chemical cues alone allow NMR only in the post-emergence period **(B)**. Circle and bars, respectively, represent mean and standard error of the mean; non-nestmate (in red), nestmates (in blue). ns, not significant pairwise comparisons, *significant pairwise comparisons ($p < 0.05$), which means effective NMR.

TABLE 2 | The clypeus pattern of the lures significantly affects aggressiveness, but only in the "only visual" treatment.

Source of variance	*F*-value	df1	df2	Sig.
Treatment	8.040	2	214	<0.001
Lure category	68.576	1	214	<0.001
Clypeus pattern	5.407	2	214	0.005
Treatment × Lure category	9.830	2	214	<0.001
Treatment × clypeus pattern	10.248	4	214	<0.001
Lure category × clypeus pattern	4.531	2	214	0.012

Results from GLZ show that in both the pre-emergence and post-emergence phase, the aggressive response of foundresses also depends on the clypeus pattern of the lures. The model also shows that this is not affecting NMR. Sig. = p-value.

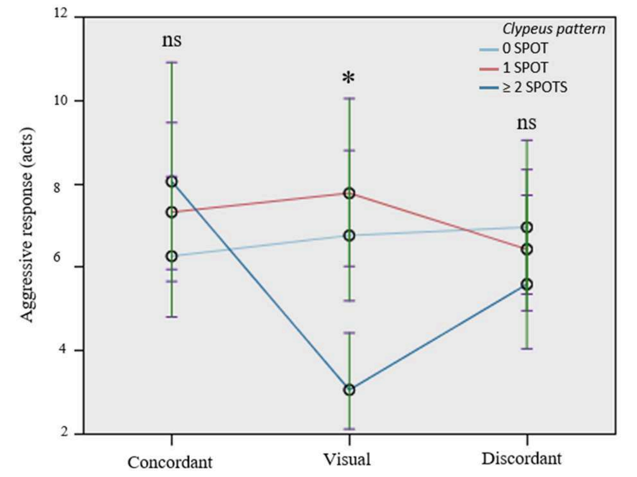

FIGURE 3 | The clypeus pattern of the lures significantly affects aggressiveness, but only in the "only visual" treatment. Circle and bars, represent mean and standard error of the mean; the clypeus pattern of the lure is depicted by lines of different colors (no spot = light blue, one spot = red, two or more spots = dark blue). ns, not significant pairwise comparisons, *significant pairwise comparisons ($p < 0.05$).

pattern had a significant influence on aggressive response, with the pattern with two or more spots being less attacked than the two other patterns (no spot and one spot, $p = 0.025$ and 0.004, respectively) (**Table 2**). The significant interaction between treatment and clypeus pattern also revealed that the treatment affected how lures with different clypeus patterns were treated. In particular, the clypeus pattern showed a significant effect only in the "only visual" experiment ($F = 16.171$, df = 2.214, $p < 0.001$), while no differences exist in the concordant and discordant treatment ($F = 2.216$, df = 2.214, $p = 0.112$; $F = 1.284$, df = 2.214, $p = 0.279$, respectively) (**Table 2**; **Figure 3**). Wasps with 0 spot and 1 spot pattern were significantly more attacked than those with 2 or more spots ($p < 0.001$), while no significant difference existed between no spot and 1 spot patterns ($p = 0.124$). The significant interaction between lure category and clypeus pattern (**Table 2**) showed that, while in all cases non-nestmates were significantly more attacked than nestmates, this difference was reduced and lost significance for lures with 1 spot pattern.

DISCUSSION

Our results show that the relative importance of NMR cues of the two sensory modalities, visual and chemical, changes according to colony phase in *P. dominula* wasps. In the early phase of the colony cycle, before workers' emergence, foundresses favor visual over chemical cues in the NMR recognition process. Conversely, in a more advanced colony stage, when many individuals are on the nest, workers rely on the chemical cues rather than on the visual ones to discriminate among nestmates and foreign individuals.

This difference in the importance of visual and chemical cues for NMR between the two conditions can be explained by the interplay between the features of the two sensory modalities and the different colony contexts across the season, which translates into different reliability of NMR cues in the two different colonial phases. Before emergence of workers, *P. dominula* colonies are composed only by foundresses and colony size is thus relatively small (ranging from 1 to 10 individuals, usually around 2–4; Reeve, 1991). After the emergence of workers, colony size rapidly increases up to dozens of wasps. It is thus conceivable that visual cues might be sufficiently variable and easier to be used in the first but not in the second phase. Indeed, the variation in the color patterning of the clypeus, the only visual cue so far shown to be perceived and used in intraspecific communication outside the sexual context (reviewed in Cervo et al., 2015), is limited. Actually, in many populations, a significant percentage of wasps show very similar facial pattern (Cervo et al., 2008; Zanette and Field, 2009; Green and Field, 2011), so that they can be categorized in a few classes (Cervo et al., 2008, 2015; Tibbetts and Lindsay, 2008). This suggests that the reliability of visual cues for NMR rapidly decreases as colony size increases. Moreover, reliability might also decrease because of an intrinsic cognitive difficulty for wasp brain to remember many visual patterns. The clypeal color patterning is, indeed, only partially genetically determined and seems to be affected by environmental factors, such as food and climate, during larval development (Tibbetts and Curtis, 2007; Green et al., 2012). This eventually results in large colonies having many kinds of facial patterns (personal observation), which might make the visual-cue-based NMR less effective and reliable.

Reliability of chemical cues might instead follow an opposite path. Despite the fact that a proper comparison of reliability of CHCs as NMR cues in different phases of colony life has never been done, it is conceivable that early season colonies (and thus pre-emergence ones) have a less marked colonial chemical signature than advanced stage colonies. This is suggested by the following: (i) in the pre-emergence phase, CHC profiles of foundresses are strongly influenced by individual social rank (Sledge et al., 2001); and (ii) the more homogeneous composition of advanced colonies, in terms of both physiology (for example in terms of fertility) and relatedness, compared to pre-emergence colonies. Indeed, few weeks after workers' emergence, the colony is consists of the dozens of sister workers, which share the genotype and many physiological features (above all, they are almost all unfertile or poorly fertile) (Queller et al., 2000). On the contrary, small pre-emergence colonies show a higher heterogeneity, with wider variation in the physiological status of foundresses (Pardi, 1946, 1948; Röseler et al., 1980; Röseler, 1991) and relatedness (Queller et al., 2000; Leadbeater et al., 2011), all factors that are known to affect CHC individual profile (Bonavita-Cougourdan et al., 1991; Sledge et al., 2001; Dapporto et al., 2004b, 2005). The colonial chemical signature is the product of a template shared by all individuals thanks to social interactions (contacts, trophallaxis) and through the nest material (Signorotti et al., 2015). It is likely that the more homogeneous conditions of late-season colonies allow the production of a more marked and reliable colonial chemical signature, while in pre-emergence colonies, individual level heterogeneity might somehow reduce inter-colony differences in the chemical profile. Moreover, the internal reference template might also be weaker in foundresses than workers. This is reasonable, as foundresses start to create their templates on their natal colonies (months before colony founding) and then update it during their life (Dapporto et al., 2004a), so that several months separate template formation and its use in NMR, while for workers, only a few days separate template formation from its use in NMR.

The finding that chemical or visual cues were not sufficient to allow NMR in pre-emergence and post-emergence colony phases, respectively, does not mean that they had no influence in the decisional process of wasps. Indeed, when both visual and chemical stimuli were coupled on the sample lure in a discordant combination (with both nestmate and non-nestmate cues on the same lure), wasps were not able to distinguish nestmates from non-nestmates, even if the relevant set of cues (i.e., visual in pre-emergence experiment, chemical in the post-emergence experiment) was still present. This suggests that the discordance in provided cues weakened the NMR process, highlighting a possible cross-modality sensory integration.

Overall, our results show, for the first time, a dynamic change in the cues used for NMR by *P. dominula* colonies. We highlight a few possible limitations of our study. Our study compared two very different periods, to cover the wide variation in contexts that colonies experience. This means that colonies differed under several aspects. First, to respect the natural conditions, focal wasps subjected to NMR trials were foundresses in the first experiment and workers in the second. This implies that age (foundresses are several months old, while workers are only days/weeks old) or caste-related differences (foundresses are reproductive individuals while workers are not) could have played a role. While we believe that age is unlikely to have an influence, as *Polistes* wasps are able to perform NMR within a few hours after emergence (thus well before the time at which they were tested) (reviewed in Signorotti et al., 2015), we cannot discard the hypothesis of differences between castes in the NMR system as it has been shown in a social bee (Wittwer and Elgar, 2018). Previous studies offered mixed evidence for the related species *Polistes fuscatus*: one study documented differences in recognition between queens and workers (with queens having a more restrictive acceptance threshold than workers against unrelated conspecific intruders; Fishwild and Gamboa, 1992), while a more recent experiment found no evidence of such a queen–worker variation in recognition (with workers showing similar ability in familiar recognition compared to queens; Injaian and Tibbetts, 2014). As *P. dominula* shows a very weak caste differentiation, which is mainly behavioral rather than physiological (Pardi, 1948; Reeve, 1991), we believe that the hypothesis of hard-wired castal differences in recognition system is unlikely. However, we believe that this needs to be tested by simultaneously evaluating the cues used for NMR by queens and workers in the same colony stage.

Second, in pre-emergence colonies, we recorded the response of the only individual present on the nest (colonies were founded by two females, one of which became the nestmate lure), while in post-emergence experiments, the response of many workers present was recorded. While analytically this does not represent a problem, as comparisons were internal to colony phase and colony id, one might speculate that group dynamics influence more the use of one sensory modality than the other. Future studies should thus investigate the use of visual and chemical cues for NMR focusing on the same individual (thus having the same phenotype, i.e., foundress or worker) through their entire life cycle in contexts where reliability of cues differ (i.e., the same queen before and after worker emergence, or the same wasps in nest by altering experimentally phenotypic variation in NMR cues). Similarly, it would be interesting to evaluate whether colony size (number of wasps) alone affects the sensory modality used in NMR. Our experiment, which compared two extreme opposite situations (small pre-emergence colonies vs. bigger post-emergence colonies), did not allow the disentanglement of the two factors, colony size and colony stage, which are usually correlated; future experimental work should test colony of the same stage with a different colony size, i.e., pre-emergence colonies with variable number of foundresses or post-emergence colonies with variable number of workers.

Our results indicate that the treatment influenced in a similar way the overall level of aggressive response in both experiments, with treatments involving both chemical and visual stimuli together (concordant and discordant treatments), which overall evoked more aggression, and the treatment presenting only chemical stimuli, which evoked the lowest levels of aggression. Moreover, visual cues alone elicited more aggression (toward both nestmates and non-nestmates) than chemical cues alone (significant result in the post-emergence phase and close to significance in the pre-emergence phase). These results are not surprising, as concordant and discordant treatments had lures that represented more biologically significant stimuli, as they had both chemical cues and visual cues (wasp heads). For the same reason, also the “only visual” treatment, which had wasp heads as lures, represented a more biologically relevant stimulus than the chemical only lures, in which a filter paper was covered with chemical cues extracted from the wasp body surface. It is not surprising thus that resident wasps were more aggressive toward what has a greater resemblance with a potential intruder, as already shown and discussed in this species (Cappa et al., 2016).

Our study also highlighted a significant effect of clypeus pattern of wasps in mediating aggressive behavior. The role of clypeus pattern in shaping aggressive interaction in *P. dominula* is highly debated. Several studies, performed in the non-native range of distribution (North America), showed that facial markers convey information about the competitive ability of an individual to potential opponents, which would thus use these visual cues to assess the agonistic abilities of potential rivals and minimize the time and costs of interactions, especially during the nest founding stage contests (Tibbetts and Dale, 2004; Tibbetts and Lindsay, 2008; Tibbetts et al., 2010). In particular, wasps having two or more spots are supposed to advertise a higher agonistic ability, and should thus be less challenged than wasps advertising lower agonistic ability (Tibbetts and Lindsay, 2008). However, this hypothesis has been repeatedly tested in the native range populations (Spain and Italy, for example) and no evidence has been found: facial patterns do not correlate with social dominance or other indicators of strength or health (Cervo et al., 2008), nor do they seem to be used in aggressive interactions (Branconi et al., 2018). Finally, it seems that facial pattern has no adaptive value in the wild (Green et al., 2013). Intriguingly, in this study, we report, for the first time in a population of the native range (Italy), that the kind of clypeus pattern of opponents influences, to a certain extent, the aggressive reaction of resident females of *P. dominula*. In particular, when chemical cues are ruled out, wasps with two spots are less attacked than wasps with one or no spot. This is in accordance with what was suggested by Tibbetts and Lindsay (2008), as wasps with two spots might advertise their greater competitive ability.

Our findings also highlight that visual cues mediate two different facets of social recognition in *P. dominula* wasps. First, they allow to recognize nestmates from non-nestmates, likely through a process of familiar recognition (i.e., wasps do recognize certain patterns as familiar, see below). Second, they might allow a mutual assessment during aggressive interactions. Our results also show that the two processes coexist, as in the only visual experiment foundresses were able to recognize non-nestmates from nestmates and, at the same time, their aggressive response was also influenced by the kind of clypeus pattern.

Overall, our results shed light on a possible involvement of facial pattern in shaping aggressive encounters also in the population of the native range. However, we also show that these effects are superimposed by chemical cues since they are evident when only visual cues are presented (only visual treatment). This suggests that the importance of clypeus pattern as advertisers of wasp agonistic ability, at least in this population, might come into play only under specific circumstances, as when information provided through other sensorial channels is unreliable.

We believe that our results provide several interesting insights, both at the taxon-specific level (*Polistes* paper wasps) and at a wider perspective. First, at the taxon-specific level, we demonstrated that visual cues alone can allow NMR in specific context, i.e., in small groups, which are interestingly those in which eusociality evolved in wasps. In this case, this type of social recognition can be considered familiar recognition. This is the first such finding for polistine wasps, as so far NMR based on visual cues has been shown only for hover wasps (Baracchi et al., 2015). The relevance of visual cues in NMR opens interesting perspectives on the highly debated topic of the use of visual communication in *Polistes* paper wasps, in which the absence, presence, and different level in the use of visual cues are demonstrated in different species and populations for a wide range of social recognition processes, from familiar recognition to gender recognition (reviewed in Cervo et al., 2015; Cappa et al., 2016). Moreover, we unexpectedly found that chemical cues alone are not sufficient in pre-emergence to allow accurate NMR, which suggests that the long-lasting tenet that NMR in social insects is governed by chemicals not necessarily holds true for all species in all contexts.

Under a wider perspective, our results also suggest an important concept. We argue that NMR can take the shape of familiar recognition in small groups and of NMR in large societies. It is possible that, within animal groups that shift from being small associations to large societies, group members first learn to recognize individual by familiarity (and possibly by individual recognition, also suggested for *P. fuscatus* and other social insects; Tibbetts, 2002; d'Ettorre and Heinze, 2005) and then, when colonies grow, shift to NMR. In the latter, individuals are recognized as nestmate if they bear the specific colonial label (Gamboa et al., 1986; Dani, 2006; van Zweden and d'Ettorre, 2010). This is a drastically different process from what occurs in the perennial large societies of many ants, termites, and bees, in which colony foundation by swarming or colony fission prevents the "small society" phase, thus precluding the possibility of a familiar recognition.

In conclusion, our results demonstrate an underestimated plasticity in the mechanisms of social recognition within the same species across different contexts. The same kind of social recognition (i.e., NMR) can be based on very different cues (visual and chemical ones) in different social environments and, at the same time, the same cues (i.e., clypeus patterns) can mediate two different social recognition processes (NMR and, putatively, mutual assessment of agonistic ability). Ultimately, this highlights the limitations of communication studies focusing on a single and/or specific context, life stage, or phenotype. Unfortunately, there is a dramatic lack of replication studies in animal (and especially insects) communication studies. Given its biological features and the easiness of manipulation, *P. dominula* will certainly represent a fruitful model to assess these topics in the future.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

AC and RC planned the experiment. AC, FC, IP, LP, LD, and RC performed the experiment and analyzed behavioral data. AC analyzed data and wrote the paper. All authors read, commented, and finally agreed on the MS.

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Nestmate Recognition in Social Insects: What Does It Mean to Be Chemically Insignificant?

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Social insects use the blend of hydrocarbons present on their cuticle to efficiently distinguish nestmates from aliens. Intruders must therefore find a strategy to break the recognition code in order to exploit the colony resources. Twenty years ago, the concept of "chemical insignificance" was introduced to characterize those parasites bearing almost no recognition cues on their cuticle, thus appearing chemically undetectable to their hosts. In some cases, intruders do possess cuticular hydrocarbons, but these are present in lower amount with respect to their hosts and/or they belong to different classes than the hydrocarbons typically used as recognition cues. We propose to include these cases under the label of chemical insignificance. If chemical compounds are absent on the cuticle of the intruder, or if they are produced but not perceived by the host (e.g., below the detection threshold), or if they are perceived but not meaningful, in all cases the result is identical: the profile of the intruder appears chemically neutral; thus, it is irrelevant for the host. We also discuss the consequences of producing low amounts of cuticular hydrocarbons, given that their original function is to act as a barrier against desiccation. Clarifying the concept of chemical insignificance will help unify terminology and stimulate interdisciplinary research efforts involving simultaneous investigations of chemical profiles, behavior, and physiology to elucidate the proximate and ultimate mechanisms characterizing the co-evolutionary arms race between hosts and parasites.

Keywords: ants, bees, wasps, cuticular hydrocarbons, social parasites

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Twenty years after the concept of "chemical insignificance," characterizing insect parasites bearing almost no recognition cues on their cuticle, was introduced (Lenoir et al., 1999, 2001), several comparable chemical adaptations have been described in the literature, often using different terminology. In this perspective, we briefly review the use of these different terms and propose to include all of them under the label "chemical insignificance." Using multiple terms to address a phenomenon that appears to have similar adaptive significance in different species can hinder the comprehension of both its underlying proximate mechanisms and evolutionary pathway.

CUTICULAR HYDROCARBONS AS RECOGNITION CUES

Social insects use the complex blend of hydrocarbons present on their cuticle for nestmate recognition (Lenoir et al., 1999; Dani, 2006; Blomquist and Bagnères, 2010; d'Ettorre and Lenoir, 2010). This mixture is composed of different classes of hydrocarbons, such as linear alkanes, methyl-branched alkanes, and alkenes, with a chain length typically ranging from 20 to 40 carbon atoms, although in some cases, heavier hydrocarbons have been detected. The cuticular hydrocarbon (CHC) profile is species-specific, meaning that different species show qualitatively different CHC mixtures, characterized by a species-specific combination of compounds (Bagnères and Wicker-Thomas, 2010). The CHC profile is also colony-specific, implying that within a given colony, the "colony odor" is generally uniform, but different colonies of the same species show quantitatively different profiles, i.e., different relative proportions of the same hydrocarbons. The homogeneity of the colony odor is maintained by exchanges via trophallaxis, allogrooming, and/or contact with the nest material (Lenoir et al., 1999).

DEFICIENCY OF RECOGNITION CUES IN CALLOWS AND PARASITES

Remarkably, some individuals do not show the typical species/colony CHC profile. Newly enclosed (callow) social insect workers usually possess very low amounts of CHCs at emergence and can be experimentally transferred from one colony to another, even a different species, without eliciting an aggressive response from adult workers (e.g., ants: Errard, 1994; wasps: Lorenzi et al., 1999; bees: Breed et al., 2004). Lenoir et al. (1999) termed this lack of chemical recognition cues "chemical insignificance." Young workers then go through a process of chemical integration by synthesizing CHCs and also by acquiring them via interactions with colony members (allogrooming, trophallaxis) and contacts with nest material (Bos et al., 2011). This ontogeny of the CHC profile can last several days; for instance, workers of the ant *Aphaenogaster senilis* acquire a CHC profile typical of adults in about 20 days (Ichinose and Lenoir, 2009), while it takes about 4 days in *Bombus terrestris* (Sramkova and Ayasse, 2009). *Polistes* wasps acquire the adult CHC profile in about 3 days, during which they increase drastically the total amount of the hydrocarbons but also incur a qualitative change: the proportion of branched hydrocarbons increases at the expense of that of linear hydrocarbons and relatively long-chain hydrocarbons increase at the expense of short-chain ones (Lorenzi et al., 2004a).

The concept of chemical insignificance has been extended to social parasites (Lenoir et al., 1999, 2001) that need to infiltrate and be tolerated into a host nest. Lacking conspicuous recognition cues may help both remaining undetected when entering a host colony and facilitating the acquisition of the colony odor from the nest material and the hosts. Chemical insignificance works effectively if the host recognition system is based on the undesirable-present (U-present) model, and not on a point-by-point label/template matching. In the U-present model, rejection by the discriminator is elicited only by the presence of additional/odd cues on the intruder, either different compounds or higher amounts of some compounds that are also present of the cuticle of the discriminator (Guerrieri et al., 2009; van Zweden and d'Ettorre, 2010). The underlying perceptual mechanism may act at the level of the antennae (Ozaki et al., 2005; Ozaki and Hefetz, 2014) or at higher levels (e.g., antennal lobes: Guerrieri et al., 2009; Brandstaetter et al., 2011), although these two models are not mutually exclusive (Bos and d'Ettorre, 2012). Therefore, when the incoming individual bears no detectable cues, theory predicts that the individual will not be rejected. A reduced amount of recognition cues on the cuticle (with respect to residents) has the same effect as the absence of cues, providing that the amount does not reach the detection threshold of the discriminator. Data on the detection threshold of social insects are very scarce. We know that ants can detect very low amounts of hydrocarbons, namely, a total CHC extract equivalent of 10^{-4} workers in *A. senilis* (Ichinose and Lenoir, 2010), and can discriminate different concentrations of the same hydrocarbon, with one concentration as low as 0.3 ant equivalents (*Camponotus aethiops*, Di Mauro et al., 2015). Foundresses of the paper wasp *Polistes dominula* start reacting aggressively when presented with lures carrying the two-thirds of the total surface extract obtained from one wasp, while the reaction to one-third of the extract does not differ from the reaction to the solvent alone (Cini et al., 2009), suggesting that paper wasps might have a higher detection threshold than ants.

CHEMICAL INSIGNIFICANCE AND CHEMICAL TRANSPARENCY

Not all the classes of hydrocarbons are good candidates to act as recognition cues. There is evidence that methyl-branched alkanes and alkenes are more important than linear alkanes in the recognition process (review in van Zweden and d'Ettorre, 2010). Consequently, a cuticular profile characterized by linear alkanes and a reduced number/amount of the other hydrocarbon classes may also be chemically undetectable in the context of nestmate recognition. The production of a profile characterized by the presence of hydrocarbons that are not used as recognition cues has been termed "chemical transparency" (Martin et al., 2008). For example, adults and eggs of the social parasite *Vespa dybowskii* show a chemical profile dominated by alkanes and alkenes, while methyl-branched compounds are only present in traces (<1%). These methylated compounds, which act as recognition cues in hornets, are instead present in high proportion in the host species *V. simillima* (41%) (Martin et al., 2008). Larvae of the paper wasp social parasite *Polistes sulcifer* appear to use a similar strategy, as their cuticular profile shows a higher relative proportion of linear alkanes but a lower proportion of branched and unsaturated hydrocarbons than the profile of host larvae, *P. dominula* (Cervo et al., 2008). Similarly, the parasite *Polistes atrimandibularis* larvae show higher proportion of linear alkanes compared to their host larvae (Elia et al., 2018). In these examples, even if the total amount of CHCs is similar in hosts and parasites, the relative amount of key compounds is significantly lower in parasites (see also **Table 1**). Therefore, these parasites produce a chemical mixture that is "invisible" to the host in the recognition context and therefore does not elicit aggression. We propose that these cases of so-called chemical transparency or chemical neutrality should be included under the broader label of chemical insignificance. Moreover, chemical insignificance, in the form of reduced total amount of CHCs, might compensate for potentially "visible" traits in the parasite CHC profile, for instance, the presence of relatively short-chain CHCs (which are in principle conspicuous) in *P. atrimandibularis* parasites before they invade a host nest (Uboni et al., 2012), or the "imperfect" mimicry of the cuckoo wasp, *Hedychrum rutilans* (Kroiss et al., 2009) (**Table 1**).

Another aspect to be considered is the hydrocarbon chain length. Very-long-chain hydrocarbons are characterized by low volatility and thus are relatively more difficult to perceive for the discriminator by olfaction, which is the usual way of hydrocarbon detection (Brandstaetter et al., 2008). Producing a CHC profile shifted toward long-chain hydrocarbons could be a form of chemical insignificance (Lambardi et al., 2007). However, this does not seem to be the general case, at least for the examples listed in **Table 1**, in which—if anything—we observe sometimes the opposite trend, with parasites having a profile characterized by lighter compounds than their hosts. An intriguing case is that of parabiotic ant species, in which a large-scale comparison highlighted that associated species show significantly longer CHCs and higher proportions of methyl-branched alkenes (very rare compounds among insects) and alkadienes than non-associated species, suggesting that the evolution of interspecific amicable associations is linked to a shift toward higher chain lengths (Menzel and Schmitt, 2012).

WHY PRODUCE HYDROCARBONS?

If the lack of CHCs facilitates integration into the host colony, one should expect the totality of parasites being denuded of these cues. Yet, we observe the majority of them producing at least a minimum amount (e.g., 20% of the amount found in the hosts, Kroiss et al., 2009; Uboni et al., 2012). The original function of hydrocarbons is protection against desiccation and also pathogens and toxins; hence, parasites might be obliged to produce them. However, not all hydrocarbon classes are suitable to prevent desiccation. Linear alkanes (n-C20-C40), especially with long chain, are better than other classes in limiting dehydration as they are solid and in a relatively impermeable state at temperatures $<40^{\circ}$C, while they begin melting and become more water permeable at higher temperatures (Gibbs and Pomonis, 1995). When alkanes are blended with unsaturated CHC (alkenes) or methylated (branched) alkanes, the resulting blend is characterized by a lower melting temperature than a blend composed exclusively of linear alkanes, and thus it becomes more permeable and less effective as a waterproofing barrier (Gibbs and Rajpurohit, 2010). The cuticular layer of social parasites is typically relatively rich in linear alkanes with respect to their hosts (**Table 1**), which may increase cuticle impermeability and counterbalance the overall low amount of CHCs. However, the relationships between CHC composition and their physical properties may be more complex: the physical properties of complex blends, such as those that characterize the cuticular layer of insects, are poorly understood and their waterproofing properties are rarely studied (Gibbs and Rajpurohit, 2010). Even the presumptive correlation between amount of hydrocarbons and waterproofing (Hadley, 1981) does not always hold. For instance, several species of *Cataglyphis* desert ants, which forage at high temperature, have considerably lower total amount of CHCs (about ¼) than *Myrmica* ants, which live in humid boreal forests, but *Cataglyphis* ants lose less water than *Myrmica* ants due to a lower transpiration rate (Lenoir et al., 2009). The profile of *Cataglyphis* is characterized by a higher proportion of saturated compounds and a lower proportion of alkenes compared to that of *Myrmica*. Therefore, physiological adaptations may contribute to water balance and counteract effectively a low amount of CHCs, as well as morphological adaptations, such us a thicker cuticle in parasites compared to their hosts (Cervo, 1994). In addition, chemically insignificant parasites might compensate for the costs of a higher potential for desiccation through specific behavioral strategies. For instance, they might spend more time than their hosts in less dry, shadowy, parts of the nest, or increase the ingestion of liquids, e.g., by soliciting trophallaxis from the hosts. Preliminary field data on the parasitic wasp *P. atrimandibularis* are consistent with this hypothesis: parasites appear to avoid direct sunlight by seeking the shade (they are less active than the hosts and rest more time behind the nest in the part not exposed to the sun), and they are more involved than their hosts in trophallactic exchanges as recipients, rather than as donors (Lorenzi, personal observation).

DISCUSSION

We show that there are at least three different ways in which parasitic insects can wear a cloak of chemical invisibility in order to facilitate the intrusion and tolerance in a host nest: (i) the total absence of CHCs, (ii) the production of a reduced amount of CHCs (below the discriminator's detection threshold), and (iii) the production of hydrocarbons that are not used as recognition cues.

In the first case, CHCs are virtually totally lacking from the cuticle of the intruder, thus preventing hosts from detecting any chemical cue that might inform them about the presence of the intruder. While this was the original observation that prompted the emergence of the term chemical insignificance (Lenoir et al., 1999, 2001) in some social parasites of ants, subsequent discoveries in other insects have offered less drastic examples of paucity of recognition cues (reviewed in Bagnères and Lorenzi, 2010). Bearing a reduced amount of CHCs with respect to the host target, the second case that we highlight is a relatively more common adaptation and may result in intruders sneaking into host nests undetected, or at least not detected enough to elicit the most violent rejection. If the amount of cues falls below or around the detection threshold of the host, the amount of aggression will be limited, as suggested by the observation that the level of aggressive responses is positively correlated to the total amount of recognition cues on lures in wasps and ants (Cini et al., 2009; Ichinose and Lenoir, 2010).

Finally, the chemical profile of intruders may be composed of hydrocarbons that are not primarily important in the nestmate recognition process. For instance, bearing a higher proportion of linear alkanes, rather than branched alkanes, is a common characteristic among social parasites. If only part of the chemical

TABLE 1 | Characteristics of the CHC profile of parasites that differ from that of hosts (calculated as "parasites *minus* hosts").

Parasite (type)	Host	Reduced CHC total amount	Proportion of CHC by class	Difference in chain-length	Reference
Polistes atrimandibularis (social parasite)	*P. biglumis*	Yes	>LA >E <BA	Lighter	Lorenzi and Bagnères, 2002; Uboni et al., 2012
Polistes semenowi (social parasite)	*P. dominula*	Yes	No	Lighter	Lorenzi et al., 2004b; Lorenzi, personal observation
Polistes sulcifer (social parasite)	*P. dominula*	Yes	>LA <BA	Possibly lighter	Turillazzi et al., 2000; Sledge et al., 2001
Bombus sylvestris (social parasite)	*B. pratorum*	Yes			Dronnet et al., 2005
Polyergus rufescens (social parasite)	*Formica cunicularia*	Yes	Almost no CHC		Lenoir et al., 2001
Polyergus breviceps (social parasite)	*Formica gnava*	Yes	Almost exclusively LA		Johnson et al., 2001
Polyergus samurai (social parasite)	*F. japonica*	Yes	Almost exclusively LA		Tsuneoka and Akino, 2012
Acromyrmex insinuator (social parasite)	*A. echinatior*	Yes	>LA	Contradictory results: Heavier than host Lighter than host	Lambardi et al., 2007; Nehring et al., 2015
Temnothorax sp. (social parasite)	*Temnothorax* sp.	No	>LA <BA	Lighter	Kleeberg et al., 2017
Formicoxenus provancheri (xenobiont)	*Myrmica incompleta*	Yes	Acquired mimicry		Lenoir et al., 1997, 2001
Ectatomma ruidum (cleptobiont)	*Ectatomma ruidum*	Yes	Same species, no qualitative differences		Jeral et al., 1997
Attaphila sp. (myrmecophilous cockroach)	*Acromyrmex octospinosus*, *Atta colombica*	Yes, *Acromyrmex* No, *Atta*	No	No	Nehring et al., 2016
Mutilla europaea (cleptobiont)	*Polistes biglumis*	Yes	>LA >E <BA	Lighter	Uboni et al., 2012
Hedychrum rutilans (parasitoid)	*Philanthus triangulum*	Yes, but also mimicry	No	No	Kroiss et al., 2009
Sternocoelis hispanus (myrmecophilous beetle)	*Aphaenogaster senilis*	No	Mimicry		Lenoir et al., 2012
Silverfish (myrmecophilous)	*Aphaenogaster senilis*	Yes	>LA <BA	Lighter	Lenoir et al., 2012

"Parasite" is used as a general term, including parasitoids, cleptobionts, and myrmecophilous species. For social parasites, we refer to the phase before host nest invasion; for hosts, we refer either to the same phase, or to unparasitized hosts.

">": larger in parasites than in hosts; "<": smaller in parasites.

LA, linear alkanes; BA, branched alkanes; E, alkenes. Cells are empty when the information is not available.

profile informs about the identity of the bearer, lacking that part is equivalent to being chemically insignificant.

It could be argued that alternative explanations exist for the observed large difference in the total amount of CHCs between social parasites and hosts. For instance, hosts may not necessarily express a minimum amount of CHCs to avoid dehydration, they could have an excessive amount of CHCs on their cuticle to serve recognition purposes (the "host chemical over-significance" hypothesis). In this view, the amount of CHCS on the social parasite cuticle, despite being lower than that of the host, might be enough to prevent dehydration. In our opinion, this hypothesis is less parsimonious than that of chemical insignificance, since CHCs are supposed to be costly to produce and therefore hosts might incur high costs for overexpressing them (e.g., d'Ettorre and Heinze, 2001; Holman et al., 2010). Moreover, the chemical over-significance hypothesis calls for different explanations even among closely related species of social parasites. Young queen of *Polyergus rufescens* social parasites have virtually no CHCs, while *Polyergus breviceps* and *P. samurai* have some but less CHCs than their hosts. Under the chemical insignificance hypothesis, the three *Polyergus* species employ the same strategy, and differ only in the how extreme their strategy is; under the chemical over-significance hypothesis, the lack of CHC in *P. rufescens* is not easily explained. Finally, there is evidence for a gradual mode of evolution of CHCs in ants (van Wilgenburg et al., 2011), which also contradicts this alternative hypothesis.

A total lack of recognition cues, a diluted chemical profile, or the lack of hydrocarbons relevant in recognition processes may be parsimoniously interpreted as parasite adaptation that serve the same function: sneaking into host nests undetected. In a review about terminology, von Beeren et al. (2012) suggest the term "chemical hiding" when an organism shows the total absence of recognition cues or the presence of cues below the discriminator detection threshold (thus not detectable), as opposed, for instance, to "chemical masquerade" (detected but misidentified as uninteresting entity, eliciting no reaction) and "chemical mimicry" (resembling an interesting entity, such as a nestmate). However, one can hide by resembling something else; therefore, we believe that a functional approach might be more practical than focusing on terms. Furthermore, "chemical insignificance" is now a term widely employed in the social insect literature, and it might be more difficult to replace it than to clarify its meaning by including the three functional categories presented here.

The different ways of being chemically insignificant are not mutually exclusive: for instance, invading females of the social parasite *P. atrimandibularis*, as well as females of the cleptoparasite *Mutilla europaea*, are chemical insignificant by producing only 20% of the total amount of CHCs compared to their host, and also express primarily linear alkanes, at the expense of the more informative branched alkane component of the profile (Uboni et al., 2012). Chemical insignificance offers a protection against detection, but it might also be a way to facilitate the acquisition of host colony odor (Lenoir et al., 2001; Lorenzi et al., 2004b; Lorenzi, 2006). This hypothesis stems from the observation that newly eclosed social insects are typically chemical insignificant (i.e., poor in recognition cues) and acquire the colony odor with time (Lenoir et al., 1999). Chemical insignificance may be not completely effective: *Polyergus* queens, as well as *P. atrimandibularis* parasites or *Hedychrum*, are regularly attacked when they infiltrate the host colony, which means that other cues elicit the reaction of the hosts. For instance, visual cues might be used by residents (e.g., visual cues have a role in interactions among nestmates in wasps) as well as tactile cues, which might help distinguish a chemically insignificant parasite from the background.

Finally, what is especially surprising is that, at least in social insects, chemical insignificant social parasites share the same habitat as their hosts: they live in the same colony and are exposed to the same physical stressors (e.g., solar radiation, heat, dryness) as their hosts, and therefore are likely to share similar physiological requirements to cope for water loss (Lorenzi, 2006). If CHCs act as a protective barrier, chemical insignificant parasites have to find a solution to reduce the impact of these stressors. One testable hypothesis is that behavioral modifications (e.g., minimizing direct exposure to sunlight, reducing general activity, and increasing introduction of liquids) may compensate for the deficiency of CHCs.

The data are currently too scanty to draw conclusions, yet preliminary measures of total CHC quantities and environmental parameters suggest that the association between the amount of CHCs and dehydration risk may be mediated by modifications involving other traits, such as behavior and physiology. We hope that clarifying the concept of chemical insignificance by using a functional approach will help both unify terminology and broaden interdisciplinary research effort involving simultaneous investigations on chemical ecology, behavioral adaptations, and water balance physiology.

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ML and PE conceived and wrote the paper.

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What Drives Diversity in Social Recognition Mechanisms?

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Recognition allows animals to categorize social partners and differentiate among them in adaptive ways. Recognition systems are a fundamental component of social interactions, and a major goal for behavioral and evolutionary ecology is to understand the factors that influence the diversity of traits involved in social recognition across species and contexts. Here we argue that recognition is best understood as the interaction between a population of diverse senders and receivers with different perspectives and experiences. Receivers vary in the extent to which they agree on the category membership of senders and this variation is a key parameter that may explain the diverse evolutionary pressures shaping recognition systems. High receiver agreement (e.g., sex recognition) should favor uniformity in signals and innate recognition templates in receivers, while low receiver agreement (e.g., neighbor recognition) should tend to favor diversity in signals and flexible learning in receivers. Further, variation in how specifically receivers categorize senders may constrain the evolution of signals that need to function for multiple audiences. It remains an open question how receivers integrate multiple signals of different types of social categories. By framing recognition systems in a population context we hope this perspective will help spur new efforts to model and empirically investigate the mechanisms underlying the diversity of recognition systems across animals.

Keywords: individual recognition, class-level recognition, template, signal evolution, communication, social cognition, phenotypic diversity

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INTRODUCTION

Animals need to categorize individuals they encounter to navigate their social environments. They do so using signals or cues produced by those individuals, and a social category consists of all of the individuals that are grouped together by a receiver. Examples of social categories include "my offspring" or "my territory neighbor." While receivers of a given species often have shared social categories of interest, they may disagree on who belongs in those categories (e.g., different territory holders will have different neighbors). They may also differ in which social categories they attend to at any given time. Our argument in this paper is that variation in receiver agreement about sender categorization has the potential to explain a great deal of the diversity in mechanisms of social recognition, in terms of variation in signals, and the mechanisms by which receivers classify signal variation.

A recognition system involves an interaction between a sender and a receiver of a communication signal or cue (**Box 1**; Sherman et al., 1997; Mateo, 2004). Recognition occurs when a receiver's perception of a signal or cue matches an internal representation ("template") of that signal or cue in the receiver's nervous system (Stoddard, 1996; Ryan and Rand, 2001; Bee, 2006). The receiver responds based on how it has categorized the signal, and the nature of the receiver's

BOX 1 | Components of recognition systems.

1) **Production:** senders produce a signal or cue
2) **Perception:** receivers perceive a signal or cue and compare it to a template of that signal or cue in their nervous system.
    a) *Template acquisition*: templates of signal or cue properties are either innate or learned
    b) *Categorization*: the cognitive association of templates with relevant social categories
3) **Action:** receivers respond appropriately based on whether perception of a signal or cue matches their template.

BOX 2 | Frameworks for classifying diversity in social recognition.

Context approaches

Recognition is commonly classified based on context or function. There are many examples of terms used to classify recognition based on context, including recognition of *neighbors*, *nest-mates*, *group members*, *parent-offspring*, *mates, kin*, *castes*, etc. Researchers using this terminology are often interested the adaptive value and social context of recognition. Kin recognition, in particular, has its own rich history of research and terminology for different mechanisms of recognition (reviewed in Penn and Frommen, 2010).

Cognition-based approaches

These approaches are primarily motivated by an interest in understanding receiver cognitive abilities and social intelligence. A point of considerable discussion and disagreement in this literature is the criteria needed to demonstrate *individual recognition* as opposed to a more generalized *class-level recognition* (e.g., Halpin, 1986; Gheusi et al., 1994; Tibbetts and Dale, 2007; Johnston, 2008; Steiger and Müller, 2008). The distinction hinges on the specificity and complexity of an animals' cognitive representations of social partners and often seems aimed at finding examples that most closely approach our own capacities for individual recognition as humans. Class-level recognition is generally defined as occurring when a receiver recognizes a sender as belonging to a particular "class" or social category (e.g., neighbor vs. stranger, offspring vs. un-related young), but does not discriminate between individuals within a class (e.g., if a parent cares for all of its offspring equally). Individual recognition is commonly defined as when receivers can discriminate between individuals, even within a class, however varying definitions of individual recognition persist.

Recognition systems approaches

Recognition involves communication between a sender and a receiver of a communication signal or cue (see **Box 1**). Classifying the diversity in traits that underlie recognition can allow for the identification of recognition mechanisms that are shared across contexts and taxa as well as the evolutionary forces that shape these traits (Sherman et al., 1997; Tibbetts and Dale, 2007).

response enacts fitness consequences for both the sender and the receiver (Reeve, 1989; Sherman et al., 1997; Liebert and Starks, 2004). Diverse mechanisms enable recognition across animal species, reflecting a variety of evolutionary trajectories of recognition system evolution. For example, signals vary from relatively uniform to highly variable among individuals (e.g., Buckley and Buckley, 1970; Gerhardt, 1991; Tibbetts, 2004). Further, receivers vary in the extent to which they rely on learned vs. innate templates in recognizing and responding to signals, and they vary in the specificity and complexity of their cognitive representations of social partners (Tibbetts and Dale, 2007; Miller and Bee, 2012; Wiley, 2013; Yorzinski, 2017). A remaining challenge for research on social recognition is to identify sources of selection that shape the evolution of traits that compose recognition systems.

Historically, research on social recognition has focused on the function and contexts of recognition, as well as the cognitive abilities of receivers in recognizing social partners (**Box 2**). Theoretical treatments of the evolution of recognition signals often consider average receivers in specific social contexts (e.g., Crozier, 1986; Beecher, 1989; Johnstone, 1997; Dale et al., 2001; Sheehan et al., 2017), but social recognition occurs among populations of diverse senders, and receivers with different experiences and motivations. This population perspective is important because receivers often vary in their responses based on, for example, morphology (Gill et al., 2013) or experience (Tanner et al., 2019), and this variation can impact signal evolution. Here we take a recognition systems approach (**Boxes 1**,**2**) to explore how variation in receiver agreement about how to categorize senders provides an important and over-arching framework for explaining the diversity of recognition traits in both senders and receiver across a range of social and sexual contexts. Specifically, the extent to which receivers agree on how to categorize sender phenotypes is a key variable that may explain similarities and differences among diverse recognition systems.

RECEIVER AGREEMENT FRAMEWORK

We consider two axes of variation in agreement of sender categories for populations of receivers. First, receivers vary in the extent to which they agree on the category membership of senders. In other words, do all receivers in a population agree that senders expressing trait X belong to category Y? At one extreme, all receivers will agree on the category membership of senders. For example, adults of sexually dimorphic species express traits that are indicative of male or female. Red cardinals can be unambiguously classified as males by conspecifics. Though male and female cardinals may differ in how they respond to the category of "male," and females could be further interested in assessing fine variation in red coloration, the category of "male" is agreed upon. Situations in which receivers have high agreement on membership in social categories would include recognition of species, sex, age-class, and caste (**Figure 1**). These are objective social categories in that membership in such categories is intrinsic to senders and not dependent on receiver experience.

At the other extreme, receivers have low to no agreement on the category membership of senders. Consider a neighborhood where many individuals are defending territories across a landscape. Territory holders often recognize neighbors and only respond aggressively to strangers, producing a phenomenon called the "dear enemy effect" (Fisher, 1954; Wilson, 1975; Tumulty, 2018). Even if every territory holder recognizes neighbors, they won't agree on which individuals belong to this social category. In other words, one receiver's neighbor is

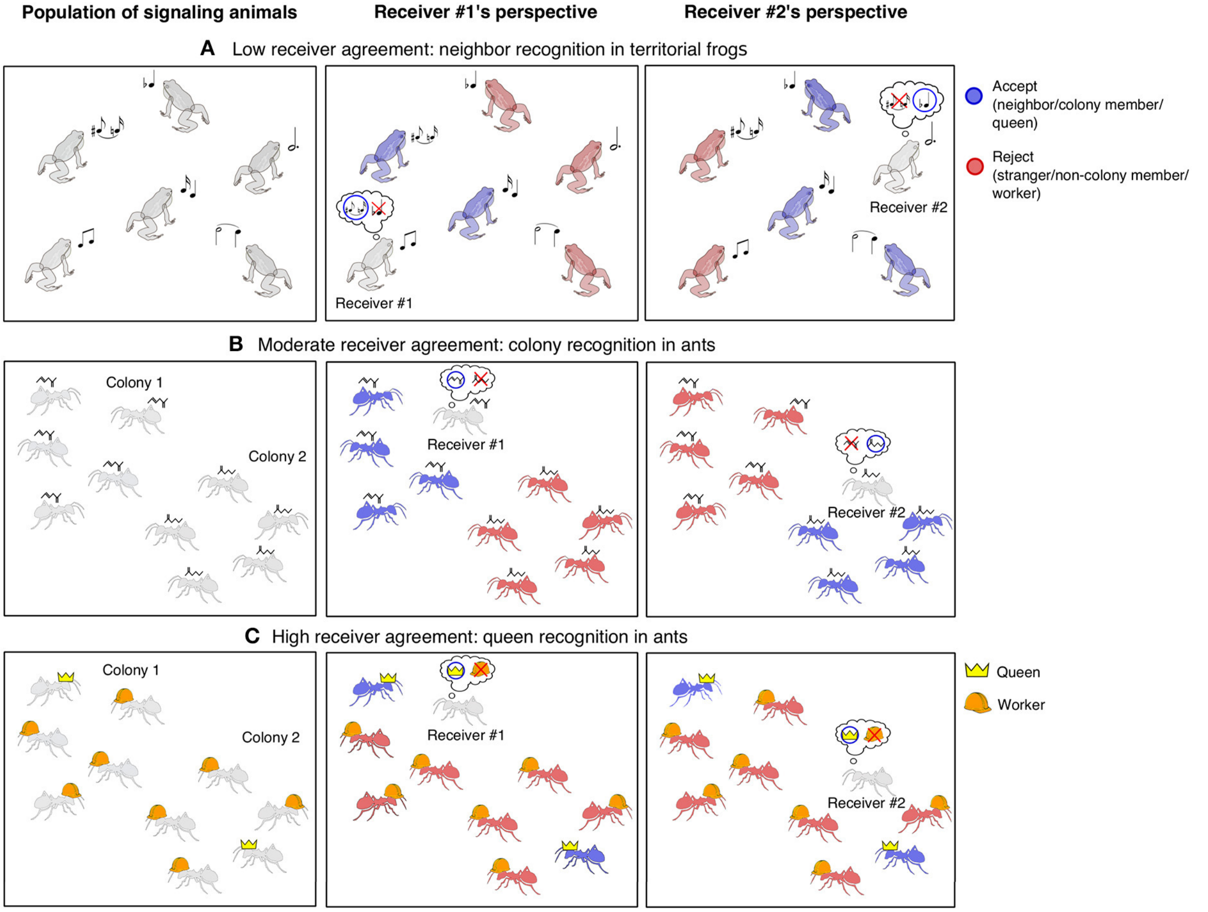

FIGURE 1 | Among recognition systems, receivers vary in the extent to which they agree on the category membership of senders. **(A)** In a population of territorial frogs, individuals are categorized as either neighbors, or strangers depending on their spatial proximity to a particular receiver. Receivers thus have low agreement on category membership and individuals must have individually distinctive traits (e.g., calls represented by musical notes in this example) to be recognized as members of such egocentric categories. Note: for simplicity, only two calls are shown in the receiver thought bubbles. **(B)** All members of a colony of ants share the same cuticular hydrocarbon profile, which differs between colonies. The decision of whether to accept an individual as a colony member or reject it as a non-colony member depends on the colony to which a receiver belongs. At the level of the population, there are moderate levels of receiver agreement because all members of the same colony agree on who belongs in which categories, but members of different colonies disagree. **(C)** Whether an individual is a queen (represented by a crown) or a worker (hard hat) is an objective social category that does not depend on the perspective of a receiver, resulting in high agreement about category membership among a population of receivers.

another receiver's stranger. Therefore, a population of receivers will have low agreement on which members of the population belong in the neighbor vs. stranger categories. As such, it is not possible for a single phenotype to be associated with "neighbor" or "stranger" (**Figure 1**). Situations with low receiver agreement include recognition of mates, parents, offspring, and neighbors. These are egocentric social categories because assignment to a particular category depends on the experience of a particular receiver and not on intrinsic features of the sender.

The second axis of variation we explore is the specificity of categories that receivers attend to. The specificity of social categories can be considered hierarchically based on the number of individuals that belong to categories (Wiley, 2013). The most general category includes all individuals of a given species, while the most specific is a single individual. Intermediate levels of specificity range from "my offspring" which may include a handful of individuals, to "colony member" which may include hundreds or thousands of individuals in the case of social insects. Different receivers in a population may be interested in different levels of this continuum simultaneously. For example, a parent may need to categorize its offspring as individuals, or perhaps more generally as "my offspring" vs. "not my offspring." While this need for categorization may be true of all parents in a population, non-parent receivers would not need to know whose offspring are whose, but they may still care to know that a sender is immature. In this example, a given juvenile may be categorized as "my offspring" by its parent, "not my offspring" by an unrelated parent, or more generically as "juvenile" depending on the receiver in question. Senders may produce signals or cues that that allow for recognition and categorization across multiple hierarchical layers due to the variation in specificity of recognition among receivers within a population.

IMPLICATIONS TO THE EVOLUTION OF RECOGNITION MECHANISMS

Understanding the extent to which receivers agree about the categorization of senders provides a novel framework for thinking about both the mechanisms and evolution of recognition systems. Here, we focus on situations in which senders and receivers have shared evolutionary interests, but we also briefly highlight how conflicting interests between senders and receivers may shape recognition systems.

Receivers Vary in the Extent to Which They Agree on Category Membership

Consequences for Senders

When receivers agree on categorization, similarity among senders should be favored as uniformity within a category will facilitate recognition across receivers (**Figure 2**). For signals with a genetic basis, such uniformity can be the result of stabilizing selection within categories and divergence between categories. There are countless examples of signals that have likely been shaped by recognition in this way, including calls used for species recognition in gray treefrogs (*Hyla versicolor*, Gerhardt, 1991), throat color signals of mating strategy in side-blotched lizards (*Uta stansburiana*, Sinervo and Lively, 1996), visual signals of sexual receptivity in female sticklebacks (*Gasterosteus aculeatus*, Rowland et al., 1991), pheromones used for sex recognition in crayfish (*Procambarus clarkii*, Ameyaw-Akumfi and Hazlett, 1975; Hazlett, 1985), and cuticular hydrocarbons used for caste recognition in ants *(Aphaenogaster senilis*, Ruel et al., 2013). For signals that are more plastic, uniformity can be achieved through homogenization among category members. Colony-specific odors used for nest-mate recognition in social insects develop in just this way (Gamboa et al., 1986; Breed et al., 1988; Breed and Stiller, 1992). Both of these processes would result in senders within a category having similar traits (i.e., homogenous subgroups; Barrows et al., 1975). While there may be many inadvertent cues of category membership that are produced because of a shared genetic or developmental basis of particular categories (e.g., males and females often differ in body size for reasons unrelated to sex recognition), if senders benefit from being recognized, selection should favor the elaboration of signals that facilitate recognition.

If receivers do not agree on which individuals belong to categories, they must recognize social partners using individually distinctive phenotypic traits and associate these traits with social categories (**Figure 2**). For example, as discussed above, there cannot be inherent phenotypic traits associated with categories such as "neighbor" or "stranger," because such categories are egocentric and dependent on receiver experience. Very few territory owners will share the same neighbors, so if territory owners are to recognize neighbors, they must do so using individually distinctive phenotypic traits of neighbors (**Figure 1**). Individuals may often vary due to genetic and developmental differences unrelated to recognition, but provided being recognized is beneficial, selection should favor greater phenotypic diversity in senders when receivers do not agree on category membership. Such diversity can result from negative frequency dependent selection in which individuals with rare, recognizable phenotypes have higher fitness than individuals with common phenotypes (Sheehan and Tibbetts, 2009; Tibbetts et al., 2017). Signals in paper wasps (*Polistes fuscatus*; Sheehan and Tibbetts, 2010), house mice (*Mus musculus*; Sheehan et al., 2016), and humans (*Homo sapiens*; Sheehan and Nachman, 2014) show patterns of selection for identity signaling, as do signals across species of swallows (Medvin et al., 1993), bats (Wilkinson, 2003), marmots (Pollard and Blumstein, 2011), and penguins (Aubin and Jouventin, 2002).

Consequences for Receivers

Similarity among senders means that receivers can have shared templates that are used to recognize members of relevant categories (**Figure 2**). Where template similarity is favored, innate templates are possible. This is because the meaning of a signal of category membership is stable across generations. In such situations, learning is not required each generation and coevolution between senders and receivers can select for signals that are reliably associated with category membership and templates that enable receivers to respond appropriately to such signals. Innate templates of agreed-upon categories are likely extremely common in nature. Some examples include recognition of cues of sexual receptivity in rats (*Rattus norvegicus*, Landauer et al., 1977) and mammary pheromones in newborn rabbits (*Oryctolagus cuniculus*, Schneider et al., 2016).

Recognition templates must be learned when receivers disagree on category membership of senders. This is because such categories are egocentric with respect to a particular receiver (**Figure 2**). Templates could be acquired through imprinting if senders are encountered early in life in a predictable context and their membership in that category does not change within a lifetime (e.g., parent imprinting in ducks and geese, reviewed in Shettleworth, 2009). However, if category membership changes throughout a lifetime, templates must continue to be acquired, or updated. For example, the identity of a neighbor is seldom fixed during a lifetime and is determined based on where an animal establishes a territory and who settles nearby. Further, territory occupancy may change over time, and residents often have multiple neighbors (Stoddard, 1996; Wiley, 2013). In these situations, animals must be able to form multiple recognition templates over the course of their lives, and potentially update and modify these templates. For example, territorial white-crowned sparrows (Brooks and Falls, 1975; Zonotrichia albicollis, Baker et al., 1981), and bullfrogs (*Rana catesbeiana*, Bee and Gerhardt, 2002) likely learn to recognize neighbors by habituating to their neighbors' vocalizations and territory locations, allowing them to discriminate between multiple neighbors and create new neighbor templates if new neighbors arrive.

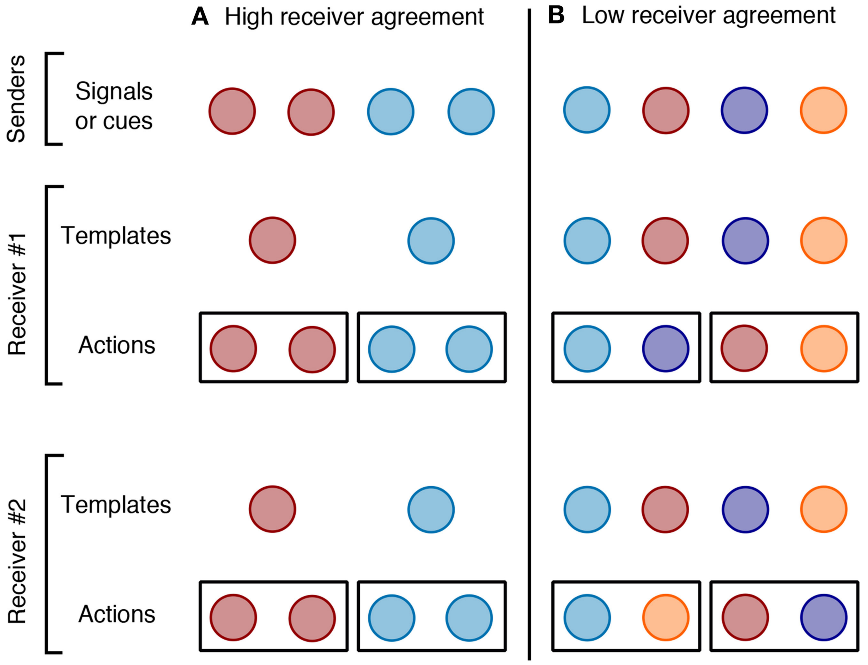

FIGURE 2 | The representation and grouping of sender phenotypes (colors) as (1) signals or cues produced by senders, (2) templates in a receiver's nervous system, and (3) receiver actions (e.g., accept or reject). Each dot represents a hypothetical individual, different colors represent different phenotypic signals or cues used for recognition, and boxes surround individuals who are treated as belonging to the same social category by a receiver's actions. **(A)** when receivers agree on category membership (e.g., sex recognition), senders share signals or cues associated with a certain category, and receivers possess one template per category that can be used to recognize any individual that they encounter who is expressing that signal or cue. Recognition is not dependent on receiver experience so all receivers should associate the same phenotypes with the same categories. **(B)** When receivers disagree on category membership (e.g., neighbor recognition), signals or cues and templates are individual-specific but actions need not be. Receivers in population can share the same categories but they disagree on who belongs in those categories so they must use individually-distinctive traits to assign senders to categories.

When Senders and Receivers Have Conflicting Interests

The predictions outlined above are for situations in which senders and receivers both benefit from recognition. However, when senders do not benefit by being recognized, we would generally expect the opposite patterns to emerge. When receivers agree, selection may favor diversity in senders to inhibit recognition and favor learning in receivers. This process seems to underlie apostatic selection as prey evolve polymorphisms to avoid detection by predator search images (Bond and Kamil, 1998). Conversely, when receivers disagree about category membership, senders could cause confusion by evolving uniformity. This may be important in instances of paternity confusion. Fathers are interested in differentiating their own offspring from other offspring, but detection would be costly to extra-pair offspring, selecting against identity signaling by chicks (Kempenaers and Sheldon, 1996).

Receivers Vary in the Specificity of Categorization

Consequences for Senders

A population of receivers that are interested in different levels of social categories may constrain the evolution of signals. Many receivers attending to and responding to signals or cues associated with social categories impose selection on senders, and the benefit of communicating information that all receivers could agree upon at one level (e.g., "male of my species") could favor uniformity that may then constrain the diversity of traits at another level of categorization (e.g., "individual" or "neighbor"). Senders can potentially solve this problem in two ways. First, senders can adjust the types of signals they provide in different contexts or life stages if the relative importance of different groups of receivers and the costs and benefits of recognition also vary across life stages and contexts. The plumage patterns of royal terns (*Thalasseus maxima*) provides an interesting example. Mobile young are reared in large colonies making it potentially challenging for parents to locate their offspring. Chicks have highly variable plumage coloration and patterning (Buckley and Buckley, 1970) that facilitates offspring recognition by parents (Buckley and Buckley, 1972). However, at later life stages, juveniles, non-breeding adults, and breeding adults all have distinct plumage patterns that are associated with their age and breeding status (Buckley and Buckley, 2002). Whereas, receiver disagreement appears to be an important force shaping plumage identity signals in young terns, adult breeding plumage is relatively uniform among senders within a given social category, suggesting that the receiver agreement in categorization is high at later life stages. The second potential solution is to use multiple signals components or modalities to convey information. We would expect this solution to arise in situations where different receivers are interested in different levels of categorization simultaneously. This solution is analogous to the "multiple messages" hypothesis in animal communication (Gerhardt, 1992; Johnstone, 1996), which posits that different signal components convey different types of information about the sender. For example, many vocalizations encode information about identity, age, and sex, such as alarm calls of yellow-bellied marmots (*Marmota flaviventri*, Blumstein and Munos, 2005) or bleats of giant pandas (*Ailuropoda melanoleuca*, Charlton et al., 2009). Scent marks also frequently contain multiple tiers of social information, as has been shown for black rhinoceros (*Diceros bicornis*, Linklater et al., 2013) and house mice (Mus musculus, Hurst and Beynon, 2004; Sheehan et al., 2019). The role that populations of receivers play in shaping these signals by responding to different levels of social information in these signals simultaneously is very much an open question.

Consequences for Receivers

Because senders are evolving to provide information to a population of receivers that will vary in extent of interest and agreement on categorization, receivers should be equipped to assess a wide range of sender phenotypes that will correspond to both innate and learned templates. This raises the question of how receivers integrate information that corresponds to multiple templates and categories when assessing a sender. An individual's identity is made up of many different categories that vary in specificity, including species, sex, and age. As reviewed above, these features can often be encoded within a single complex trait such as a vocalization, scent, or color pattern. It is clear that receivers can extract information at different levels of specificity from the same signals. For example, field sparrows use variation in song frequency to recognize both species and individuals (*Spizella pusilla*, Nelson, 1989). Further, experiments in domestic horses (*Equus caballus*, Proops et al., 2009) and rhesus macaques (*Macaca mulatta*, Sliwa et al., 2011) demonstrate that receivers sometimes integrate signals from different modalities (visual and auditory) to recognize individuals. But how do receivers integrate information about different levels of specificity in recognition? For example, when a receiver recognizes a sender as an individual, do the features that make the sender recognizable as part of broader, agreed-upon categories like species and sex form part of the template for that individual? Or are templates for different levels of specificity distinct, with receivers using simpler decision rules for discriminating broader categories and attending to more specific levels of categorization only when it is beneficial to do so? Understanding the hierarchy of receiver categorization is important to uncovering the mechanisms by which recognition systems work in the complex social settings found in real animal populations. This is currently an unresolved question, but its answer will reveal how receivers integrate information across levels of social categories.

IMPLICATIONS FOR OTHER CONTEXTS

Recognition is a fundamental component of animal lives beyond their social interactions and variation in receiver agreement may have implications to other contexts as well. Here we briefly discuss some of these potential implications. First, foraging is a context that is often mediated by communication (Bradbury and Vehrencamp, 2011). For example, aposematic animals benefit by advertising their toxicity to would-be predators and predators benefit by recognizing aposematic signals as indicators of toxicity (Rojas et al., 2015). Müllerian mimicry, whereby two different toxic species converge on the same aposematic phenotype (e.g., Kapan, 2001; Symula et al., 2001), may represent an example of selection for uniformity driven by high receiver agreement since receivers (predators) possess or develop one template for the objective category "toxic prey" and thereby impose selection on senders that are members of this category to label themselves as such with shared signals. Pollination syndromes—similar suites of shared floral traits among different species that attract the same pollinators (Fenster et al., 2004)—represent a similar example of convergent evolution of signals in response to shared receiver templates. Examples of receiver disagreement in foraging contexts are perhaps less common because there are fewer opportunities for repeated interactions between the same individuals. But, by generalizing our framework to repeated interactions that an individual has with members of a given species that it forages on, floral constancy may represent an example of low receiver agreement. Floral constancy occurs when pollinators demonstrate short-term learned preferences for certain flowers as a result of associating a reward (nectar) with signals from that particular flower (e.g., color, odor) (Schiestl and Johnson, 2013). This preference is egocentric since not all foragers will express the same preference (Heinrich, 1976), and it is thought to be a source of selection for floral distinctiveness (Schiestl and Johnson, 2013). A second context in which this receiver agreement framework seems relevant is in the importance of learning mate choice preferences. When preferences are genetically determined, a population of receivers agree on the traits that indicate high quality mates, and thus impose stabilizing or directional selection on those traits. However, in some cases, receivers can have low preference agreement if preferences for traits that are experienced early in life are learned, a phenomenon termed "sexual imprinting" (Verzijden et al., 2012). This phenomenon can be a source of selection for signal diversity, for example, by helping to maintain reproductive isolation between closely related sympatric species (Verzijden and Ten Cate, 2007) or leading to the stable coexistence of polymorphisms under some conditions (Yang et al., 2019).

CONCLUSIONS AND FUTURE DIRECTIONS

Here, we have argued that recognition is a multiparty process in which senders and receivers communicate with each other in a population. We argue for taking into account the diversity of receiver perspectives, as doing so can provide new insights into the evolution of traits mediating recognition. Notably, this perspective applies to a range of social categorization and recognition contexts, providing an overarching framework to consider how receiver behavior and sender phenotypes interact and coevolve. Whether or not receivers agree on how to categorize a sender should determine the selection pressures shaping patterns of diversity in signals. At the same time, agreement among receivers opens of the possibility of innate templates, whereas disagreement requires learned templates. Not all receivers will be concerned with identifying senders based on the same sorts of social categories, which may constrain the evolution of sender traits. This framework highlights the need for future models of recognition systems to consider the diversity of receiver perspectives and for empirical studies to probe the constraints on sender traits and receiver integration of innate and learned templates when assessing senders.

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All authors contributed to the development of the ideas and writing and editing of the manuscript.

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REVIEW
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Social Recognition and Social Attraction in Group-Living Fishes

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Social aggregation is a widespread and important phenomenon among fishes. Understanding the questions of why and how aggregations form and are subsequently maintained is a central goal for behavioral ecologists. Research in this field has shown that aggregations are typically structured, non-random associations. This indicates that fish are able to differentiate between potential group-mates and that this ability mediates their association preferences, and, ultimately, the composition of their groups. In this review, we examine the characteristics that influence the expression of social attraction among fishes, before going on to describe the recognition mechanisms that underpin social attraction. Finally, we highlight a number of outstanding questions in the field with a view to generating a more complete understanding of social aggregation in fishes.

Keywords: grouping, shoaling, schooling, familiarity, relatedness, fish

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INTRODUCTION

Social attraction describes the tendency of animals to approach and interact with conspecifics and is a basic mechanism underlying the formation of groups. Fundamental to social attraction is the need for animals to recognize conspecifics on the basis of cues arising from those individuals, so-called ‘social recognition.’ Here we define social recognition as the identification of conspecifics to a resolution that allows animals to mediate the social interactions that occur between them. In this way, animals are able to tailor their responses to the individuals that they encounter according to recognized characteristics. Such characteristics (including sex, age, coloration, and behavior) have been shown to be determining factors in the emergence of various social structures like dominance hierarchies and territorial assemblages, among others. Recent developments in both experimental and theoretical work have provided much greater insight into both the underlying mechanisms and functional consequences of social recognition, prompting us to synthesize this information here.

In this review, we focus primarily on social recognition in the context of group-living, with a particular focus on shoaling. Fish provide a fascinating opportunity to study both the functional and mechanistic underpinnings of social recognition and group choice decisions. Not only are fish the most speciose vertebrate order, but they span almost the entire spectrum of social organization and life-histories, ranging from the vast oceanic aggregations of some pelagic species, to the small, coherent groups of territorial fish. Additionally, fish have been used extensively for the study of social behavior, meaning that there is a rich literature in relation to social recognition and its role in determining social attraction. It has been estimated that over half of known fish species shoal at some point during their existence. Some shoal only during vulnerable, early life stages, while others live in groups throughout life. Based on this estimate, there are in excess of ten thousand species of fishes for whom shoaling represents a fundamentally important strategy that provides them with wide-ranging benefits (summarized in Ward and Webster, 2016).

The specificity to which fish are able to discriminate varies considerably, both within and between species. Social aggregations of fish tend to be dominated by, or even made up exclusively of, a single species, which implies at least the ability to distinguish between their own and other species, and a tendency to be socially attracted to conspecifics. Beyond this, fish are capable of making fine-scale assessments of potential social partners according to diverse phenotypic criteria that, either in isolation or in combination, serve to shape their association patterns. Further, fish are known to bias their behavior in favor of kin and familiar individuals, requiring a more specific form of social recognition. As individuals spend more time in close association, and as the complexity of social interactions increase, there is the potential for ever greater specificity to discriminate between animals, including the ability to recognize individuals.

In this review, we describe and discuss the current knowledge of social recognition and its manifestation through social attraction in group-living fishes. We begin by outlining the characteristics that influence social attraction in fishes, before moving on to consider the underlying mechanisms and sensory bases of recognition. Finally, we propose potential future work that might be done to resolve ongoing questions within this field.

CHARACTERISTICS MEDIATING SOCIAL ATTRACTION

Social recognition encompasses a wide range of different specificities, from basic categorizations of animals all the way through to individual recognition. Generally, the specificity of recognition capabilities relates to the ecology of the animals and the complexity of their social environment. For species such as herring and sardines that live in large schools comprising thousands or even millions of fish, there is perhaps little value in the ability to make fine-scale discriminations between individuals. By contrast, species of fish, including some cichlids and damselfish, that spend extended periods of time interacting repeatedly with a small number of conspecifics may derive important benefits from the ability to discern individuals.

We can characterize the process of recognition as occurring in three sequential steps (Sherman et al., 1997). The initial stage involves the production of cues by an individual. Various terms have been used to refer to this individual, including 'signaler' and 'sender.' Since cues may derive involuntarily through physiological processes, rather than as intentional efforts at communication, we subsequently refer to the cue-producing individual as the 'sender.' The second stage involves the detection of cues by another individual, whom we refer to as the 'receiver.' During this stage, the receiver references the sender's cues against a series of criteria, often referred to as a recognition template (Mateo, 2004). The sophistication of the receiver's ability to recognize the sender's cues depends both on the quantity and quality of the information contained in those cues, and on the complexity of the receiver's recognition template. The third and final stage of the sequence occurs in the form of a behavioral response by the receiver toward the sender. When the receiver detects the cues of a sender for the first time or following a period of separation, it may alter its behavior, biasing its response positively, for instance, associating with the sender, or negatively, for example through aggression. As recognition is a continuing process, if the sender and receiver are already in proximity, the receiver may not adapt its behavior toward the sender unless it perceives novel cues.

Broadly, recognition entails a receiver detecting cues from a sender and allocating the sender to a pre-existing category. These so-called class-level distinctions can enable simple differentiation between conspecifics and heterospecifics or more complex discrimination between kin and non-kin, or between familiars and non-familiars. Indeed, more sophisticated recognition may be achieved sequentially. After a receiver first determines that the individual in question is a conspecific, they may subsequently determine that it is a relative. Further, recognition need not be binary (e.g., 'kin' or 'non-kin'). Instead, individuals may recognize graded levels of kinship (e.g., 'sib,' 'half sib,' 'parent' or 'offspring'). If senders are allocated to multiple recognition classes, it may be recognized by the receiver as both kin and familiar (Frommen et al., 2007b). In some species, the ability to make class-level distinctions may be augmented by the ability to recognize particular individuals. In this case, the receiver learns the sender's characteristics and links those to a specific and unique identity. Among the many different species of fish that have been studied in the context of social recognition, there are examples occurring from the most basic, class-level discrimination all the way through to specific individual recognition.

Species-Level Recognition

One of the most basic forms of recognition involves the ability to discriminate conspecifics from heterospecifics. In binary choice tests, which are often used to examine the association preferences of shoaling species, focal fish that are presented with a choice between a group of conspecifics and a group of heterospecifics tend to show a strong preference for conspecifics (Keenleyside, 1955; Hemmings, 1966; Kinoshita, 1972; Sisler and Sorensen, 2008). The functional benefits of associating preferentially with conspecifics are wide-ranging and include access to pertinent social information and enhancement of anti-predator benefits (Ward and Webster, 2016).

Long range detection and attraction toward conspecific cues plays a crucial role in the settlement of, among others, social coral reef fishes. The life histories of such species involve larval dispersal after hatching. Following a period of pelagic feeding on plankton, the developing fish navigate toward appropriate reef habitat and then identify and home in on the cues of resident conspecifics who have already settled there (Sweatman, 1983, 1988; Booth, 1992; Atema et al., 2002; Dixson and Jones, 2018). In addition to the benefits of social grouping, the presence of conspecifics is an indicator of habitat suitability (Lecchini and Nakamura, 2013).

Social attraction is not, however, the only force that acts to drive the formation and maintenance of shoals. In addition to this active preference, passive assortment may occur so that fish self-organize into conspecific groups on the basis of similarities in habitat preferences, swimming speeds and activity synchrony. Indeed, passive assortment via these forces can also drive the formation of mixed-species groups that share these characteristics (Krause et al., 2005; Killen et al., 2017). Nonetheless, such groups are known to fragment along species lines when under threat of predation (Wolf, 1985). Moreover, mixed species groups can be less cohesive and less aligned than single species groups and social information may flow less readily between heterospecifics than between conspecifics in some mixed species shoals (Ward et al., 2018). It seems likely that an active preference for associating with conspecifics works alongside passive assortment in shoal formation, and that both are important in maintaining shoal coherence.

Within-Species Recognition

While shoaling fish are strongly socially attracted to conspecifics, they may not be equally attracted to all such. Some elements of social recognition are concerned with the identification of fixed traits, such as kinship, while others relate to other qualities that are not intrinsic to the individual expressing them and which may change over the lifetime of that individual. The ability to navigate the social environment effectively often demands that individual fish are attuned to a suite of co-occurring characteristics in group mates and which allow them to adopt the appropriate social response in light of these.

Aspects of appearance and behavior, and the interaction between them, mediate social attraction and association preferences across a range of characteristics. Below, we will discuss within-species recognition on the basis of various attributes, such as sex, size, relatedness and familiarity.

Sex

Sex can influence shoaling behavior in a range of different ways, including directly, by influencing sociability and the expression of shoaling preferences, and indirectly, due to sexual dimorphism and differences in habitat preference and activity synchrony between the sexes. Some species of shoaling fishes show strong patterns of sexual segregation in their social behavior. In guppies, the larger females show a pronounced tendency to shoal in same sex groups, potentially as a mechanism for reducing harassment by males (Griffiths and Magurran, 1998; Darden and Croft, 2008; Richards et al., 2010). By comparison, males show a much-reduced social tendency (Griffiths and Magurran, 1998). However, even among species that do not show pronounced sexual dimorphism, shoals may be segregated to some extent by sex. For instance, male and female minnows use different parts of their habitat even outside the breeding season, leading to assortment by sex (Griffiths et al., 2014). Different preferences may be expressed according to context. In sub-adult threespine sticklebacks, fish preferred to associate with the opposite sex under low predation threat, but changed the preference in favor of same sex fish when predation risk was greater (Rystrom et al., 2018). The shoaling preferences of individuals is also mediated by the sex of the choosing fish. For example, female zebrafish prefer larger shoals over a smaller alternative shoal (Ruhl and McRobert, 2005). By contrast, in the cichlid species, *Neolamprologus pulcher*, females prefer smaller shoals than males (Reddon et al., 2011). These choices likely represent a trade-off between the greater anti-predator benefits provided by larger shoals against considerations of competition for reproductive success or the greater opportunities for advancement in rank offered by smaller groups. Finally, the composition of shoals can often relate to the availability of potential shoaling partners. For instance, in seasonally breeding species, such as threespine stickleback, mixed-sex shoals fragment as breeding territories are established by males, leading shoals to be comprised primarily of adult females at these times (Vickery et al., 1988; Fitzgerald et al., 1992).

Size and Body Length

Shoaling fish typically express a preference to associate with conspecifics of the same size and shape as themselves in both the laboratory and in the field (Krause et al., 1996a,b; Peuhkuri, 1997; Ward and Krause, 2001; Ward et al., 2017; Kelley and Evans, 2018), potentially on the basis of forming phenotypically matched groups that maximize the anti-predator advantages of shoaling through the confusion effect or through the costs of behavioral asynchrony between differently sized fish (Theodorakis, 1989; Aivaz and Ruckstuhl, 2011). In the specific case of smaller fish avoiding larger conspecifics, there are also potentially advantages to minimizing the costs of competition. Since fish of the same size and species tend to travel at the same speed, this active preference is again bolstered by passive assortment in the formation and maintenance of groups.

Patterning and Body Coloration

Among species where there are multiple color morphs, individuals often prefer to associate with conspecifics that share the same patterning and coloration as themselves (Engeszer et al., 2007; Ledesma and McRobert, 2008; Snekser et al., 2010). As well as fixed differences in color, fish are capable of expressing different color patterns according to the local light environment and background. In Western rainbowfish, *Melanotaenia australis*, individuals acclimated to a dark background showed a strong shoaling preference for individuals also expressing the same dark coloration as themselves (Rodgers et al., 2010). By doing this, fish simultaneously maximize the predator confusion effect and reduce their per capita risk through the oddity effect (Landeau and Terborgh, 1986; Krakauer, 1995). Interestingly, this may also explain why fish sometimes join conspecific shoals characterized by a high degree of phenotypic homogeneity regardless of their own phenotype (Cattelan and Griggio, 2018).

Health and Parasitism

Aside from size and color, fish also assess the health of potential shoaling partners. This may be on the basis of externally visible indicators of parasitism, such as the spots of dark pigmentation that indicate infestation by some trematodes (Krause and Godin, 1996), the outgrowths of microsporidians (Ward et al., 2005a), or the presence of other external parasites, such as *Gyrodactylus* spp. (Croft et al., 2011; Rahn et al., 2015). Furthermore, infection and ill health may be signaled by other outward characteristics, including changes in coloration and swimming behavior (Sumpter et al., 2008). It has been shown that fish recognize these characteristics of unhealthy conspecifics and actively avoid them in shoaling contexts (Barber et al., 2000). However, the metabolic costs of mounting an immune response and the longer-term muscle wastage resulting from infection can decrease the activity levels of infected fish, positing a role for passive assortment in the exclusion of unhealthy fish from shoals (Ward et al., 2002b).

Nutrition, Competitive Ability and Dominance

Nutrition and foraging ability influence shoal choice decisions, both from the perspective of the choosing fish and in terms of the social attractiveness of potential shoaling partners. For instance, hungry and foraging fish are less likely to shoal in general (Hensor et al., 2003; Hoare et al., 2004; Schaerf et al., 2017) and exhibit different preferences for shoaling partners relative to their well-fed counterparts (Frommen et al., 2007a). Fish also show a preference for well-fed conspecifics over their leaner, hungrier counterparts, potentially as a means of either gaining access to information from successful foragers, or to reduce competition by associating with less motivated foragers (Krause et al., 1999; Sumpter et al., 2008). Similarly, minnows (*Phoxinus phoxinus*) are able to assess the competitive foraging ability of potential group mates in relation to their foraging behavior, even outside of a feeding context, preferring to associate with less competitive individuals and thereby reducing some of the competition costs of social living (Metcalfe and Thomson, 1995). Among species that form dominance hierarchies, resource gathering potential is determined to some degree by rank. For this reason, angelfish preferentially associate with subordinate conspecifics rather than dominants (Gomez-Laplaza, 2005) on the basis of previous interactions.

Behavioral Syndromes

There is now a substantial body of literature documenting the existence of behavioral syndromes or ‘personality’ in animals (Dall et al., 2004; Sih et al., 2004; Bell, 2006; Reale et al., 2007; Sih and Bell, 2008; Dingemanse et al., 2010). For instance, some individuals are consistently more sociable, displaying a greater tendency to shoal. Often, this enhanced sociability covaries with the tendency to be more shy and cautious and also less aggressive (Budaev, 1997; Ward et al., 2004b; Leblond and Reebs, 2006). Studies have found that these more sociable individuals coordinate their movements more closely with conspecifics, which is a key element of coherent shoaling (Jolles et al., 2015, 2017; Planas-Sitjà et al., 2018). Perhaps in line with this, Cote et al. (2012) reported that western mosquitofish prefer to join shoals comprising sociable conspecifics over those made up of less sociable individuals. However, a test on threespine stickleback reported a preference for shoals of bolder individuals, even though such individuals are usually less sociable (Harcourt et al., 2009). This might be explained by research on guppies, *Poecilia reticulata*, in which shoals of bold individuals and shoals of both bold and shy individuals performed a foraging task more efficiently than a shoal composed only of shy individuals (Dyer et al., 2009). Despite these important individual characteristics, the social environment can have a powerful mediating effect on the expression of individual behavior, resulting in the emergence of collective, group-level personality (Webster and Ward, 2011; Hamilton and Ligocki, 2012; Burns et al., 2017; Jolles et al., 2017).

Relatedness

The ability to recognize kin forms the basis for both inbreeding avoidance and the ability to behave nepotistically, that is, to bias behavior in favor of kin. Under laboratory conditions, there have been numerous studies documenting the ability of fish to recognize kin and subsequently to demonstrate an association preference for them (Behrmann-Godel et al., 2006; Griffiths and Ward, 2011; Makowicz et al., 2016). In threespine sticklebacks, the preference for kin is independent of prior social experience (Frommen et al., 2013). In some cases, this preference is mediated by sex. For instance, female rainbowfish (*Melanotaenia eachamensis*) show significant association preferences for same-sex siblings, but avoided their male siblings, suggesting that they are able to balance the benefits of kin association against the potential costs of inbreeding (Arnold, 2000). The benefits of associating with kin can range from faster growth rates to greater shoal cohesiveness and increased co-operation (Hain and Neff, 2009; Hesse and Thünken, 2014; Hesse et al., 2015; Thünken et al., 2015).

Although the ability to discriminate kin is widespread among fishes, there is relatively little evidence to suggest that relatedness plays a major role in structuring association patterns among the majority of social fish species in the wild. Nonetheless, where it does occur, the cooccurrence of close relatives within shoals is most often observed during early life stages. For instance, in coral reef fishes, which disperse following hatching to feed in the pelagic zone before returning to the reef to settle, close relatives at the same developmental phase may be seen cohabiting shortly after settling. Among humbug damselfish, within colony genetic relatedness is typically minimal, however related juveniles may be found at the same colony (Buston et al., 2009). Similarly, in the humbug’s congener, the three-spot dascyllus (*Dascyllus trimaculatus*), pairs of siblings may be found in close association following their return to the reef, suggesting that they may have traveled together during their larval development over a period of around a month (Bernardi et al., 2012). There are parallels in this pattern of early life associations among kin in guppies, where juvenile siblings associated in shoals though only in high predation environments (Piyapong et al., 2011), and in juvenile black perch, *Embiotoca jacksoni*, which associated in sib groups at a young age and directed aggression toward unrelated juveniles from other broods (Sikkel and Fuller, 2010).

The pattern of kin association occurring primarily early in life among many species might be because the advantages of kin association are greatest during vulnerable early life stages and gradually decrease as the fish age and grow. Alternatively, it may be an epiphenomenon driven by the initial proximity of members of the same brood when they are first born, or hatch, and their synchronous dispersal from the nest. Evidence of relatedness playing a role in shaping social groups of adult fish is comparatively rare. Genetic analysis of relatedness among shoals of adult guppies showed no obvious kin-structuring (Russell et al., 2004). Similar results have been reported for minnows (Bernhardt et al., 2012), cod (Herbinger et al., 1997) and salmon in the Baltic

(Palm et al., 2008) and in groups of clownfish (Buston et al., 2007). Comparatively few studies have shown evidence for kin grouping among free-ranging adult fish, although Pouyaud et al. (1999) reported the existence of relatedness as a factor shaping the shoaling of the Tilapine cichlid, *Sarotherodon melanotheron*. In another cichlid, *Neolamprologus caudopunctatus*, females, but not males, appeared to disperse from their natal nest in sibling groups (van Dongen et al., 2014). Migratory charr, however, retain some degree of kin association beyond early life (Fraser et al., 2005).

Familiarity and Individual Recognition

Familiarity is another key factor that shapes the patterns of association between fishes. We define this broadly as the recognition of and preferential biasing of behavior toward conspecifics based on prior social experience. There are different mechanistic paths that lead to the apparent social recognition of familiar individuals: one that is based on the recognition of a general, group-specific label which does not require the receiver to identify specific individual identities, and another that is founded on so-called true individual recognition. The criteria for this latter, complex form of individual recognition is that the sender's cue, the receiver's template and its response to the sender should each be unique and specific to that individual sender (Tibbetts and Dale, 2007). Most studies of familiarity in fishes have been concerned primarily with the manifestation of familiarity in terms of an association preference and subsequently with examining the functional consequences of this, rather than examining the mechanistic basis of its development. This is partly to do with the fact that, ultimately, the expression of familiarity in relation to association preferences is at least superficially similar, regardless of the mechanism at play. We discuss the mechanisms later in this review, confining ourselves for now to examples of the expression of familiarity and the benefits associated with it.

Association preferences for familiar conspecifics have been reported across a diverse range of fishes, including sticklebacks (Barber and Ruxton, 2000), guppies (Magurran et al., 1994; Cattelan et al., 2018), minnows (Griffiths et al., 2007), shiners (Farmer et al., 2004), rainbowfish (Brown, 2002), cichlids (Jordan et al., 2010b; Lee-Jenkins and Godin, 2013), salmonids (Courtenay et al., 2001), sharks (Keller et al., 2017), damselfish (Jordan et al., 2010a), and others (reviewed in Ward and Hart, 2003; Griffiths and Ward, 2011). In some circumstances, fish may even prefer to associate with familiar heterospecifics over unfamiliar conspecifics, although the functional benefits of this are unclear (Ward et al., 2003).

Associating with familiars is known to deliver a broad range of benefits, including stabilizing interactions among groups members by reducing aggression (Hojesjo et al., 1998; Seppa et al., 2001; but see Doran et al., 2019) and decreasing competition (Utne-Palm and Hart, 2000). As groups stabilize, individual members can devote a greater proportion of their time to more advantageous activities, such as foraging and mating (Griffiths et al., 2004). In fact, associating with familiars can increase foraging efficiency (Ward and Hart, 2005), potentially through greater information transfer through social networks (Atton et al., 2014), and the facilitation of social learning (Swaney et al., 2001). In addition to this enhanced foraging efficiency, shoals composed of familiar individuals are more cohesive and ordered, which may maximize the anti-predator advantages of shoaling (Chivers et al., 1995; Davis et al., 2017). Overall, the extent of the benefits enjoyed through the preferential association with familiar individuals was indicated by a study on minnows in which fish chose to shoal with familiars in preference to a larger shoal of unfamiliar conspecifics (Barber and Wright, 2001).

In addition to this group-level familiarity, some fish are also capable of true individual recognition, although this is more likely to develop in species who live in relatively stable social environments, in which they repeatedly interact with the same individuals. For instance, clownfish (*Amphiprion bicinctus*) live alongside the same individuals for much of their lives and show an ability to recognize their partner fish (Fricke, 1973). In the territorial cichlid species *Astatotilapia burtoni*, males can recognize individual rival males and infer their competitive ability (Grosenick et al., 2007). While these species may have developed the ability to recognize specific individuals due to their social system, research has also highlighted how different contexts may influence the ability to recognize individuals. Under threat of predation, performing predator inspection alongside a co-operative individual may yield benefits (Mesterton-Gibbons and Dugatkin, 1992). In territorial contexts, remembering the outcome of previous interactions may reduce future levels of aggression with the same individuals [i.e., the 'dear enemy' effect, Jaeger, 1981 (on salamanders); Saeki et al., 2018], although this may result through time-place learning rather than necessarily being individual recognition. Given the higher memory costs associated with learned individual recognition, it may be adaptive to adjust the specificity of recognition based on the context. Fittingly, research has shown that in a shoaling context, three-spine stickleback did not invest in individual recognition (Ward et al., 2009), but in a predator context (Milinski et al., 1990) and in a territorial context (Waas and Colgan, 1994), sticklebacks could differentiate between specific individuals. We consider further examples in relation to the cues and mechanisms of individual recognition in a later section.

MECHANISMS OF SOCIAL RECOGNITION AND SOCIAL ATTRACTION IN FISHES

In the previous sections, we described the characteristics upon which fish base their association preferences. However, the process by which fish detect such characteristics and use these to discriminate involve a range of different mechanisms and sensory modalities. Recognition often occurs on the basis of a template, providing a means for individuals to reference the attributes and cues of others. In some instances, however, local attraction may be mediated, at least initially, by the detection of movement. For instance, as the optomotor response develops, fish are drawn toward the movement of moving conspecifics (Lemasson et al., 2018). The relatively greater attractiveness of more mobile individuals or shoals (Pritchard et al., 2001; Gomez-Laplaza, 2006) may be one factor that induces fish to approach bolder conspecifics, which tend to be more active than their shyer counterparts. Nonetheless, the more specific categorization and identification of sympatric animals typically relies on a more formalized process of recognition.

Recognition Templates

To achieve recognition, animals must process the cues provided by another individual and compare them against a 'recognition template' (Mateo, 2004). Broadly, these recognition templates can be categorized as: context-based associative learning, phenotype matching, and learned characteristics (Bradbury and Vehrencamp, 1998). These templates provide a means of categorizing (and therefore recognizing) the various contextual, auditory, chemical or visual information gleaned from other individuals. In some instances, the recognition template may be genetically determined, meaning that the receiver has an innate ability to recognize and categorize the cue, or it can be acquired through learning. Generally, genetically determined templates use more fixed and stable cues, such as major histocompatibility complexes (or MHCs), whereas templates arising through learning or self-referencing may rely on more transient and flexible cues, such as those mediated by diet or environment. In the following sections, we discuss each type of recognition template in more depth and provide examples from research on a wide range of fish species.

Context-Based Associative Learning

Context-based associative learning is a basic mechanism by which animals can 'recognize' other individuals based on the spatial or temporal features in their immediate environment rather than based on any cue provided by an individual themselves. As a mechanism, it is likely to persist in instances where an observable environmental feature reliably correlates with identity. For instance, parent fish may 'recognize' fry or eggs within their burrow or nest as their own offspring. Given its simplicity, this mechanism can expose parents to the risk of brood parasitism (Sato, 1986; Polačik et al., 2019). However, using the same context-based mechanism, parent fish can infer from the number of cuckolders present at a spawning site the proportion of the brood they have sired, which subsequently increases or decreases the rate of filial cannibalism or parental investment (Gray et al., 2007). Again, this is a simplistic recognition template, which can increase the risk of accidentally consuming their own offspring. For these reasons, context-based associative learning may be restricted to specific situations (e.g., before offspring have hatched and become mobile) and often necessitate the integration of more complex recognition templates. For instance, the Lake Tanganyikan mouth-brooding cichlid, *Simochromis diagramma,* collects eggs into its mouth based on contextual cues (e.g., these eggs are in the vicinity of where I mated) but subsequently employ more complex methods of kin-recognition to expel the eggs of the parasitic cuckoo catfish, *Synodontis multipunctatus*, from the buccal cavity (Blažek et al., 2018).

Phenotype Matching

Unlike context-based recognition, phenotype matching potentially provides a more flexible recognition tool that can be employed in a range of contexts and generally provides a greater degree of specificity. Phenotype matching allows for recognition through the comparison of the phenotypic cues of an unfamiliar individual against a template. The template may be formed either innately (the receiver has a pre-determined ability to recognize cues), be self-referent (i.e., based on one's own phenotype: does this individual look, sound or smell like me?) (Dawkins, 1982; Holmes and Sherman, 1982; Mateo, 2004) or experience-based (often, though not always, through imprinting during an early, labile developmental stage).

Regardless of how the phenotype template is formed, these templates can either be fixed or flexible. Innate or imprinted templates tend to be fixed, whereas self-referencing can in some instances be fixed while in others allows flexibility. For instance, though self-referencing can often provide a flexible means of recognition through the use of a variable template that reflects the receiver's current, continually updating phenotype, when used in kin recognition, the receiver self-references against a recognition template based on its own genetic profile. Given that an individual's genotype will not change during its lifetime, a self-referent kin recognition template is effectively fixed.

Kin Recognition Through Phenotype Matching

In the context of kin recognition, phenotype matching relies on the use of cues that are more likely to be similar among related individuals than between distantly related or unrelated individuals. However, the formation of the kin recognition template often depends on the reproductive system of the species in question. For instance, many species that spend initial developmental periods in close proximity to kin (e.g., many nest-building species, mouthbrooders and livebearers) rely on imprinting during early life stages. Broadcast spawners, on the other hand, are less likely to encounter siblings during early life stages and may subsequently be more likely to rely on self-referencing. Research on Arctic charr, *Salvelinus alpinus,* which hatch alongside their siblings, revealed that individuals reared in isolation were not able to recognize unfamiliar kin whereas individuals reared in proximity to siblings could, indicating that the phenotype template is learned in this species (Winberg and Olsen, 1992). Similarly, zebrafish, *Danio rerio*, are capable of recognizing unfamiliar kin using chemical cues, although this ability does not develop in individuals deprived of kin odors on the 6th day post fertilization (Gerlach et al., 2008). This suggests that their kin phenotype template is learned and that this learning occurs specifically on the 6th day after fertilization. Interestingly, when researchers exposed zebrafish to heterospecific odors on this crucial 6th day, individuals did not develop a preference for heterospecific odors. Therefore, despite zebrafish relying on a learned phenotype template, there exists some innate predisposition or sensitivity to conspecific rather than heterospecific cues.

The failure to recognize kin without exposure to odors on the 6th day post-fertilization suggests that zebrafish are not able to phenotype match through self-referencing, although this may not always be the case in other species. For instance, research on African cichlid fish, *Pelvicachromis taeniatus*, found that reproductive males were able to discriminate between sisters and non-related females despite being isolated at the egg stage.

In this case, the ability of males to recognize kin must be based on an innate and self-derived olfactory template (Thünken et al., 2014). However, research on this same species found that juveniles, who are social (unlike reproductive-stage males), did not discriminate between the olfactory cues of shoals differing in relatedness when they had been raised in isolation from egg stage. Interestingly, they also found that cichlids raised in the presence of heterospecifics developed a preference for unfamiliar heterospecific chemical cues over unfamiliar conspecific cues (Hesse et al., 2012). This highlights the role of learning in kin recognition template and, in opposition to the work done on zebrafish, it suggests that there is no fixed predisposition for conspecific cues in this species. Furthermore, this work indicates that the mechanisms of phenotype matching may be dependent on life stage (e.g., social, juvenile stages vs. solitary, adult stages) or based on context (i.e., shoaling preferences vs. mate choice).

Different phenotype matching mechanisms may even be used by different individuals of the same species. Bluegill sunfish (*Lepomis macrochirus*) have a complex mating system in which males can either become a dominant breeder, meaning they court females and provide parental care, or they can become satellite males, meaning they adopt a sneaky mating strategy and provide no parental care (Gross and Charnov, 1980). As a result, offspring sired by parental males are more likely to be surrounded by kin than the offspring sired by sneaky males. Hain and Neff (2006) examined the effect of this asymmetry in nestmate relatedness on the recognition mechanisms adopted by different offspring. They found that when the offspring of parental males were given a choice between the chemical cues of unfamiliar kin and unfamiliar non-kin (i.e., the full siblings of the sneaky male offspring), they showed no association preference. However, when the offspring of sneaky males were presented with the same choice, they showed a clear association preference for the chemical cues of their siblings. Given that all offspring were reared together, this rules out the possibility that the offspring of sneaky males were using a learnt phenotype template. Instead, these results suggest that only the offspring of dominant males relied on a learnt phenotype template while sneaky male offspring used self-referencing to distinguish between kin and non-kin.

Cues Used in Kin Recognition Through Phenotype Matching

Although the examples above have demonstrated the use of chemical cues in kin recognition (as have many other studies, e.g., Quinn and Busack, 1985; Olsen, 1989; Brown et al., 1993; Olsen and Winberg, 1996; Mehlis et al., 2008), very few studies have identified which features of a chemical signature are used in kin recognition. However, research has focused on the specific chemical cues mediated by the major histocompatibility complex (MHC), which is a set of genes that control immunological recognition in vertebrates. MHC molecules function by binding to pathogen-derived peptides and displaying them on cell surfaces for the immune cells to inspect. Ultimately, these peptide and MHC complexes are shed from the surface of the cell and expelled in saliva and urine, contributing to the chemical signature of each individual (Milinski et al., 2005). Given the heritability of MHC genotypes, these MHC-mediated chemical cues are particularly useful when distinguishing between kin and non-kin given that related individuals are likely to have similar genotypes and therefore similar chemical signatures. In fact, some researchers have referred to the use of these chemical cues in kin recognition as genotype (rather than phenotype) matching, although the mechanisms are the same.

The use of MHC-chemical cues in kin recognition was demonstrated in an experiment by Olsen et al. (2002), in which juvenile Arctic charr (*Salvelinus alpinus*) preferred to swim in water containing cues from a sibling with the same MHC class II genotype as themselves compared to water of siblings with dissimilar MHC class II genotype. However, when presented with the cues of an MHC-similar non-sibling and an MHC-dissimilar sibling, focal individuals showed no preference. These results were mirrored in work conducted by Rajakaruna et al. (2006), in which juvenile Atlantic salmon (*Salmo salar*) and juvenile brook trout (*Salvelinus fontinalis*) failed to differentiate (or had no preference) between MHC-different kin and MHC-similar non-kin. These studies both indicate that while MHC class II genotypes play an important role in kin-recognition, there are likely other chemical cues, possibly derived from other components of the genotype, that aid in kin-recognition. This is bolstered by the fact that both juvenile Atlantic salmon and juvenile brook trout showed a preference for kin over non-kin when neither had similar MHC class II genotypes (Rajakaruna et al., 2006).

Interestingly, Olsen et al. (2002) also found that when Arctic charr were reared in isolation, they did not show any behavioral preferences based on MHC odors, suggesting that their recognition template is not innate or self-referent but learned. However, this may not be universally true across all fish species. Indeed, current research hints at the possibility of innate recognition through phenotype matching [e.g., cichlids (Thünken et al., 2014)], although no study to date has specifically demonstrated innate recognition through MHC-based genotype matching.

Despite the widespread use of chemical cues in kin recognition, there are also examples of fish requiring visual cues (Steck et al., 1999) or a combination of visual and chemical cues for kin recognition through phenotype matching (Van Havre and FitzGerald, 1988). For instance, Hinz et al. (2013) expanded on the work by Gerlach et al. (2008) to show that larval zebrafish required visual exposure to kin on the 5th day post fertilization in addition to chemical exposure to kin on the 6th day post fertilization to form a template for kin recognition through phenotype matching. When larvae were provided with only visual or chemical cues, or with only the cues of non-kin, they developed no preference for kin versus non-kin chemical cues. The presence of both chemical and visual cues appears to facilitate kin recognition in later life. Arnold (2000) found that Lake Eacham rainbowfish, *Melanotaenia eachamensis,* only formed weak kin recognition abilities when provided with chemical cues. However, strong kin recognition abilities were expressed more clearly when fish were provided with both chemical and visual cues (see also Le Vin et al., 2010). It is possible that by relying on a phenotype template shaped by both visual and chemical cues, individuals can increase the likelihood of imprinting on the correct stimulus. More generally, it may be that the recognition template is most accurate in the presence of multimodal cues and that recognition is bolstered when these cues coincide (Ward and Mehner, 2010).

Species Recognition Through Phenotype Matching

Thus far, we have discussed phenotype matching in the context of kin recognition. However, ample evidence suggests that this mechanism can be used at a more basic level to discriminate between conspecifics and heterospecifics. The mechanisms underlying species recognition share much in common with those that promote kin recognition in that they both rely to a large extent on cues that are intrinsic to the animals. The preference of fish to associate with conspecifics in shoal choice decisions is well established and wide-ranging among social animals, however, the question of whether the template involved in species recognition is innate, fixed by imprinting or self-referent has received comparatively little attention. As with kin recognition, the formation of the template may depend to a degree on the reproductive strategy of the species and thus the probability that young fish develop in proximity to conspecifics. For instance, striped kribs (*Pelvicachromis taeniatus*) reared in the nest of a congeneric species, the common krib (*Pelvicachromis pulcher*) subsequently showed a preference for the odors of heterospecifics over conspecifics, suggesting that the young imprinted on the fish with which they were surrounded in early life rather than self-referencing (Hesse et al., 2012). In a similar way, a study by Warburton and Lees (1996) reported that guppies that had been reared among swordtails (*Xiphophorus helleri*) subsequently showed a preference for associating with those heterospecifics. In both of these cases, the development of the recognition template appears to have been formed by early life experiences rather than being either innate or self-referent. In a parallel example, Spence and Smith (2007) found that zebrafish preferred to shoal with the color morph with which they were raised rather than individuals displaying the same color morph as themselves. With the exception of zebrafish, which scatter their eggs and provide no parental care, little research has examined the development of kin or species recognition templates in broadcast spawning fish, representing a clear priority for future research.

The studies discussed above have exemplified the way in which many species, often those that are reliably surrounded by kin during early developmental stages, use imprinting to form fixed recognition templates. However, the potential exists for mistakes to occur through imprinting, which may have severe fitness costs. For instance, Stephenson and Reynolds (2016) found that juvenile guppies exposed to conspecifics infected with the parasite, *Gyrodactylus turnbulli*, subsequently showed an association preference for those conspecifics carrying the parasite, which is likely to put them at risk of infection. Generally, however, the ramifications of incorrect species recognition in the context of shoaling are not this extreme. On the other hand, species-recognition in the context of mating is more likely to have severe consequences when individuals cannot correctly differentiate between viable mates (i.e., conspecifics) from non-viable mates (i.e., heterospecifics), especially in environments where closely related species overlap. Accordingly, Magurran and Ramnarine (2004) found that male guppies (*Poecilia reticulata*) from isolated populations were unable to discern between conspecific females and heterospecific females (*Poecilia picta*). However, males from sites where the two species live sympatrically could recognize conspecifics from heterospecifics. This suggests both the ability for species recognition mechanisms to adapt and evolve over time as well as the use of phenotype matching in the discrimination of different species.

Cues Used in Species Recognition Through Phenotype Matching

The sensory cues used in species recognition potentially varies across different fishes, however chemical cues are likely to play a major role (Levesque et al., 2011). The use of chemical cues allows a high degree of specificity. In a study using six closely-related cyprinid species, Sisler and Sorensen (2008) reported that common carp, *Cyprinus carpio*, and goldfish, *Carassius auratus*, were clearly able to distinguish conspecifics from heterospecifics. Further, the ability to detect conspecifics is lost in fish whose olfactory sense has been ablated (Sorensen and Baker, 2015). Ward et al. (2002a) demonstrated that chub, *Leuciscus cephalus,* prioritize chemical cues over visual cues to shoal with conspecifics rather than heterospecifics (European minnows, *Phoxinus phoxinus*) when the cues were presented in juxtaposition. In fact, when presented with two mixed species shoals, chub spent increasingly more time with shoals as the percentage of conspecifics increased. Given that European minnows outcompete same-sized chub in mix-species shoals, phenotype matching may be an important mechanism used by chub to enhance their foraging success by shoaling preferentially with conspecifics. Among social reef fishes, chemical cues are also used to distinguish conspecifics (Sweatman, 1988; Doving et al., 2006) and may be important in determining the patterns of aggression between heterospecific competitors (e.g., Bay et al., 2001). Coppock et al. (2016) found that among four different species of damselfish, three showed a preference for conspecific chemical cues while all four actively avoided heterospecifics cues. In this case, conspecific associations may be generated by both attractive and repulsive forces.

In addition to chemical cues, species-level recognition can be achieved using a visual, auditory or even electrical cues. For instance, weakly electric fish have species-specific electrical organ discharges, providing an electrical template for species recognition (Kramer and Kuhn, 1994). In coral reef fish, UV markings on the face and body have been found to promote species recognition (Siebeck et al., 2010). In many African cichlids, females prefer the coloration patterns of conspecific males over heterospecific males (Seehausen et al., 2008). This visual phenotype template has been proposed as a mechanism behind the sympatric speciation of these fishes, although there is evidence that species-specific acoustic calls may further aid in species recognition and sexual isolation (Amorim et al., 2004, 2008).

Flexibility in Phenotype Matching

Thus far, we have discussed phenotype matching as a recognition template mediating long-term species-level and kin-level preferences. However, phenotype matching can also provide a more flexible template used in short-term and shifting preferences. This is due to the fact that phenotype matching can provide a means of recognition using variable cues. One way in which flexibility can be achieved is by updating association preferences on the basis of recent experience. Juvenile angelfish, for example, adapt their preference in favor of associating with the color or pattern morph of the individuals that they most recently interacted with Gómez-Laplaza (2009) and a similar flexibility may underlie temporal shifts in preference for individuals with a matching color morph in rainbowfish (*Melanotaenia australis*) (Rodgers et al., 2010). In this case, the color expressed by the fish changes as a function of their environment, hence flexibility is required to enable individuals to adopt a shoaling preference according to their current phenotype. Though it seems likely that visual cues play a major role in this, it is possible that the preference may be augmented by chemical cues. For instance, although Ward and Krause (2001) found that body length matching in fish could be achieved through visual cues alone, Ward and Currie (2013) found that it could also be achieved through chemical cues alone. In these cases, fish appear to be self-referencing on the basis of continually updated cues in order to assort with same-sized individuals, which may provide important anti-predator benefits. However, the question of how a fish knows how large it is (or what color it is) and thus how to match its size (or color) with conspecifics purely on the basis of visual cues remains unknown.

The mix of chemicals contributing to a fish's chemical signature represent a continually changing representation of both intrinsic factors, such as their physiological state, and extrinsic factors, such as the animal's environment or diet (Henneken et al., 2017; Nikonov et al., 2017). These cues are known to affect association preferences with fish preferring to shoal with individuals that smell most like themselves, which clearly implicates self-referent phenotype matching as the mechanism. In particular, fish show an association preference for conspecifics that have eaten the same diet as themselves (Olsen et al., 2003; Ward et al., 2004a, 2005b). In addition to this, fine-scale differences in water chemistry among habitats also mediate shoaling preference, with fish favoring conspecifics that have occupied a similar habitat over those from a different habitat. The adaptability of this mechanism was examined by Ward et al. (2007), who reported that free-ranging sticklebacks transplanted between habitats gradually adopted a preference for individuals from their new habitat. The specific time frame involved in the shift in this preference appears to be in the order of 1–2 h (Webster et al., 2007). Although the precise nature of the chemical cues involved is not yet known, a study by Bryant and Atema (1987) reported that a change in the diet of yellow bullhead catfish, *Ameiurus natalis*, precipitated a change in urine-borne amino acids and, most importantly, a change in response toward those individuals by conspecifics. Diet quality may also influence association decisions and potentially provides a means of distinguishing between individuals on the basis of their foraging ability and determining which have valuable social information. In particular, the proportion of protein in the diet mediates association preferences, with individuals preferring to associate with conspecifics who had recently consumed a high protein diet (Ward et al., 2011). Again, the dietary cues are most likely expressed through amino acids in the urine (Kleinhappel et al., 2016).

Recognition typically involves the discrimination of multiple traits sequentially or even simultaneously. The ability to assess a third party on the basis of a suite of traits can lead to straightforward decisions when preferred traits are interlinked. For instance, kin recognition is obviously aligned with species recognition, however, in other cases, the co-occurrence of conflicting cues can give insight to the basis of association preferences. For example, in a study involving two stickleback species, the threespine stickleback (*Gasterosteus aculeatus*) and the ninespine stickleback (*Pungitius pungitius*), threespine sticklebacks associated with heterospecifics that had been fed the same diet as themselves in preference to conspecifics that had been fed with a different diet (Kleinhappel et al., 2016). Similarly, Ward et al. (2003) found that the preference of chub for conspecific shoals disappeared when they had to choose between unfamiliar conspecifics and familiar heterospecifics. In both cases, fish are capable of species recognition, yet chose to associate with heterospecifics based on the presence of different, perhaps more pertinent, cues (e.g., diet and familiarity). Ultimately, these studies demonstrate the many levels of recognition that can be achieved through phenotype matching and the subsequent complexity involved in making association decisions.

Learned Individual Characteristics – Familiarity and Individual Recognition

True individual recognition involves the ability of a receiver to associate a unique and distinct set of a attributes with the individual identity of the sender, and then to express a distinct pattern of behavior toward the sender (Beecher, 1989; Gheusi et al., 1994; Gherardi et al., 2012) see also Steiger and Mueller (2008). Consequently, this is most likely to be seen in stable groups, where individuals interact frequently and repeatedly over time. Further, it is a cognitively demanding process and it may be that at least some of the documented cases of familiarity, wherein fish bias their behavior in favor of individuals with whom they have prior social experience, may involve a more general recognition mechanism, focused on some group or population-specific cue. For individual recognition to evolve, selection must act not only upon the receiver, to be able to perceive and recognize cues from the sender, but also upon the sender itself, to produce salient and easily detected, recognizable and individually specific cues. This will likely only happen where these is a mutual net benefit to the sender in being recognized by the receiver (Tibbetts and Dale, 2007). These may be related to mediation of aggressive or agonistic encounters between members of stable groups or between nearby territory holders, for example. Where these conditions are not met, as may be the case in many ephemeral, fission-fusion shoals, there might be no pressure favoring adaptations for individual recognition, from either a sender or a receiver perspective. Instead, class-level recognition of the types discussed above may be sufficient for fish to make adaptive social decisions.

Nevertheless, there is evidence that some fish are capable of recognizing non-related individuals independently of context on the basis of prior social experience and learning. This ability is likely associated with cognitive constraints that limit the number of individuals whose identities may be learned (Fischer et al., 2014). Griffiths and Magurran (1997b) inferred individual recognition among guppies that lived in small, isolated pools during the dry season. They showed that guppies preferred to shoal with fish taken from their own 'home' pool, but only when the population of fish within that pool was lower than 40 or so. This preference was absent when the population of the pool was greater than this, and Griffiths and Magurran (1997b) suggest that this might reflect an upper limit on the number of separate identities that guppies can learn. If the recognition here were based on a class-level template of some sort, they argued, then the guppies should have displayed a preference for others from their own pool irrespective of the population size. Ward et al. (2009) used a different approach to tackle this question, determining that guppies were capable of learned individual recognition. First, they established that guppies preferred to shoal with unfamiliar groups that had experienced the same diet and environmental condition as themselves, which suggests an ability to recognize certain familiar group-level characteristics, via phenotype matching. Second, they established that guppies could differentiate between two shoals that had been maintained in the same tank as themselves (hence with the same diet and environmental cues), but that they preferred the group with whom they had directly interacted (rather than the ones who had been separated by an opaque barrier). Given that the guppies in this experiment were not closely related, it is unlikely that this preference was the result of kin recognition. Furthermore, given that both individuals had the same environmental and diet chemical signatures, it is unlikely that phenotype matching was the recognition template generating this preference. Instead, their results suggest an ability to recognize individual chemical cues based on experience with specific individuals.

A study by Griffiths and Magurran (1997a), demonstrated that this individual recognition template in guppies developed over a period of 12 days after repeated interactions with the same individuals. In discus fish, individuals were able to recognize fish with whom they had been housed for 3 months based on specific facial color patterns (Satoh et al., 2016). These studies help demonstrate that individual recognition is often the result of long learning periods. However, fish are also capable of more rapid learning. For instance, Dugatkin and Michael (1991) found that guppies could discriminate between two individuals and show a consistent preference for the individual that was more cooperative during a predator inspection trial (i.e., the one that swam closer to the predator). This preference was consistent whether focal individuals were made to choose between the pair directly after the predator trial or 4 h after the predator trial.

Various cues can be used to form an individual recognition template. In weakly electric African mormyrid fish, signature electric organ discharges (EODs) can be used to achieve individual recognition (Paintner and Kramer, 2003). In a study by Hanika and Kramer (2005), territorial males of *Marcusenius macrolepidotus* increased aggression when presented with longer duration EODs. However, this aggression dropped off when the playback EOD was from a familiar rival. This provides support for the 'dear enemy' effect and suggests that these weakly electric fish can use EODs as a template for learned individual recognition. In further support of the 'dear enemy' effect, Kohda et al. (2015) found that male cichlids, *Neolamprologus pulcher*, reduced aggression when presented with models that had the same facial color patterns as a familiar rival. In addition to the dear enemy effect, this study also demonstrates the use of visual cues in forming individual recognition templates and points to the importance of facial features in individual recognition more broadly (Leopold and Rhodes, 2010; Wang and Takeuchi, 2017; Hotta et al., 2017). Given the greater cognitive demands of individual recognition, it is likely to develop in socially stable species or in specific contexts characterized by repeated interactions with the same individuals. In fact, the ability of sticklebacks to recall familiars decayed over the course of 1–2 weeks when individuals were no longer interacting (Utne-Palm and Hart, 2000).

OUTSTANDING QUESTIONS

How Do Shoal Preferences in the Lab Shape Social Organization Patterns in Nature?

Social preferences based on recognition and active choice have been investigated experimentally using a number of approaches. Perhaps the commonest is the choice test, in which a test subject is presented with two or more stimulus fish or groups of fish and allowed to interact with both. The amount of time that it spends with one relative to the other is taken as a measure of shoaling preference, with a significant bias toward one stimulus group over the other(s) implying recognition of some trait particular to that stimulus group (e.g., Wright and Krause, 2006). Variations on this approach have been used in many of the examples discussed below. A less commonly used method of assessing social preference is one based upon self-organization. Here, a number of fish are placed together into an arena and allowed to separate into groups. Various statistical approaches can then be employed to allow the investigator to determine whether these groups are random subsamples of the larger pool of fish or whether they assorted by some factor. Different versions of this approach were used by Barber and Ruxton (2000) and Atton et al. (2014) to explore the effects of familiarity upon fish shoal composition. The shoal choice test can be criticized for presenting the test subjects an unnatural stimulus; it is unlikely that fish in the wild will ever be presented with a simultaneous choice between two perfectly different stimulus shoals, such as a shoal of large versus a shoal of small conspecifics. This is probably true much of the time, though not always. Guppies living in pools separated by shallow rapids travel in small groups that regularly meet and exchange members; in one study, Croft et al. (2003) describe encounters between shoals as occurring every 14 s on average. In a field study of golden shiners (*Notemigonus crysoleucas*), shoals met on average every minute or so (Krause et al., 2000). Hence, fishes living in high densities in small bodies of clear water might have many opportunities for near simultaneous observation and assessment of shoals, lending this assay a degree of ecological validity, at least under some circumstances. One clear advantage of choice test experiments over other assays is that, if designed rigorously, it can allow for recognition to be explicitly tested for. With further elaborations, this approach can also be used to explore the mechanisms of recognition, for example by blocking certain sensory channels or by systematically varying or manipulating sender cues. Even if we allow the criticism that simultaneous choices are unlikely to occur in nature under many conditions, the choice test still represents a powerful approach for demonstrating recognition and social preferences. Further, it allows us to examine how fish might weigh the relative importance of multiple different criteria in respect of potential shoaling partners, for instance whether they prioritize size, sex, color or other characteristics, and how they integrate these factors in making a decision of which shoal to join. Data from choice tests can be used *a priori* to derive hypotheses about shoal composition when studied under more natural conditions, or *post hoc*, to probe already-observed shoaling patterns.

Other Factors Affecting Shoal Composition

In some cases, fish shoal composition might not solely be shaped by active choices by group members. For example, the decision of an individual to join a group might reflect a lack of alternative choices, and predators may play a role in 'pruning' groups by targeting certain phenotypes. In addition, abiotic factors may also play a role. Some of the ideas discussed here are speculative but may warrant further investigation. The take home message here is that shoal structure can emerge without active choice (or in the absence of options to choose between) and researchers should be careful in assuming that the composition of fish shoals reflects decisions based upon recognition by the fish.

Many species of fish prefer to shoal with conspecifics and with groupmates of a similar body size. Such preferences may be adaptive, since predators may disproportionally target odd individuals, and costs to appearing different to the rest of the group will therefore be high. Where individuals are able to choose, we might expect them to select shoals of the same species or phenotype to their own. Shoals frequently split and reform, however, and individual fish can stray or become separated from their group. While being odd may be costly, being alone might be even more so, since many of the anti-predator asocial foraging benefits of grouping should accrue even to odd individuals. Under such conditions, it might pay a lone fish to join any group it encounters, even if it differs from the majority of fish within the group. This might explain why mixed-species groups are often numerically dominated by a majority species, with the other species occurring as minorities (Krause et al., 2000; Pavlov and Kasumyan, 2000). It would be interesting to quantify whether the predation and associated risks of lone fish are lower compared to similar fish that are odd members of mixed species or phenotypically mixed shoals. We predict that this will often be the case. We also predict that mixed species shoals will be less common, or at least more short-lived when the densities of all of the member species or phenotypes are greater, since this will afford more opportunities to encounter and shoal with matched groupmates.

Related to the oddity effects discussed above, in theory, targeting of odd individuals by predators might have the overall effect of reducing diversity and promoting greater within-shoal homogeneity. In this way, a shoal that initially contains a range of phenotypes might become more similar over time, as less common phenotypes are removed, without the need for active shoal choice or self-organization by the members of the shoal. It is unclear how important this process is in nature, and it is not clear whether such a pruning process could keep pace with changes in shoal composition as groups encounter one another and exchange individuals. A testable hypothesis here is that the actions of predators and the threat of predation should contribute to greater homogeneity in shoal composition in habitats with abundant predators.

Finally, assortment by body size might arise as a function of swimming energetics. If smaller-bodied fish have to expend more energy to keep a given pace than larger ones do, then shoals may be come segregated by size when moving and perhaps may even split. This effect may be exacerbated when fish are swimming against a current or holding station in moving water. This effect has already been described within shoals, where larger individuals tended to be in frontmost positions in the traveling shoals (Deblois and Rose, 1996; Reebs, 2001; Ward et al., 2017). This would lead to the prediction that groups of fish in faster-flowing water, and faster moving groups, would be more closely assorted by body length.

Assessing Social Recognition Mechanisms in Naturalistic Settings

Building on our previous point, assays of social attraction and recognition in the laboratory often involve the presentation of consistent stimulus cues with a high signal to noise ratio. For instance, studies using chemical cues are often undertaken using high concentrations of those cues and in the absence of other, potentially relevant cues that might be encountered by free-ranging fish. As a first step, such studies offer a reasonable means of determining whether fish are able to detect such cues and how they respond to them. However, determining how these cues influence the behavior of fish in the wild demands that greater ecological relevance is built into future experiments. One possible approach to this is to conduct experiments using water from the natural environment, including a mix of different chemical cues, and thus providing a more representative signal to noise ratio against which to measure social recognition and social attraction.

Broadening Our Understanding of the Mechanisms of Social Recognition

While considerable work has been done to elucidate the factors that shape the association decisions of group-living fishes, research into the mechanistic bases of this lags behind. The chief exception to this is in the context of kin recognition, which has been well studied. However, even in this case, work remains to be done to characterize the key attributes of the cues used. Many questions remain in regard to understanding the type and the ontogeny of templates used by fish to recognize conspecifics and to distinguish between conspecifics. Similarly, more work needs to be done to identify the sensory modalities used to differentiate between conspecifics and the salient characteristics of the cues that are used. An obvious example is how fish recognize and avoid diseased conspecifics, which has been resolved in other taxa [e.g., amphibians (Kiesecker et al., 1999), mammals (Kavaliers et al., 2005), and crustaceans (Behringer et al., 2006)].

SUMMARY AND CONCLUSION

Social recognition forms the basis of social organization. In the context of group-living in fishes, it permits individuals to distinguish between potential grouping partners and mediates differences in social attraction among them, ultimately structuring and shaping patterns of association among individuals. A combination of a basic social attraction toward conspecifics and more passive processes such as activity synchrony and co-ordination of swimming speeds are likely sufficient to explain the large, structured aggregations of pelagic fishes. Nonetheless, the groups formed by many other species are often reliant on more complex forms of recognition and, in turn, permit the development of more intricate patterns of association and social behaviors. Examples of these include groups that are structured by relatedness or familiarity, encompassing individual recognition, and which persist over extended periods of time. Our understanding of the mechanisms of social recognition, including the recognition template used by receivers and the characteristics of the cues expressed by senders, requires further research, not least in order that we might be able to predict how social fishes will adapt to future environmental challenges. Approaches that combine both detailed insights of patterns of social organization in free-ranging fishes with an understanding of the underlying mechanisms of social recognition and social attraction offer the best means to advance this field of research.

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

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REVIEW
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Kith or Kin? Familiarity as a Cue to Kinship in Social Birds

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Interacting with relatives provides opportunities for fitness benefits via kin-selected cooperation, but also creates potential costs through kin competition and inbreeding. Therefore, a mechanism for the discrimination of kin from non-kin is likely to be critical for individuals of many social species to maximize their inclusive fitness. Evidence suggests that genetic cues to kinship are rare and that learned or environmental cues offer a more parsimonious explanation for kin recognition in most contexts. This is particularly true among cooperatively breeding birds, where recognition of familiar individuals is usually regarded as the most plausible mechanism for kin discrimination. In this article, we first review the evidence that familiarity provides an effective decision rule for discrimination of kin from non-kin in social birds. We then consider some of the complexities of familiarity as a cue to kinship, especially the problems of how individuals become familiar, and how familiar individuals are recognized. We conclude that while familiarity as a mechanism for kin recognition may be more parsimonious and widespread than genetic mechanisms, its apparent simplicity as a decision rule governing social interactions may be deceptive. Finally, we identify directions for future research on familiarity as a kin recognition mechanism in social birds and other taxa.

Keywords: kin discrimination, kin recognition, cooperation, familiarity, social birds

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INTRODUCTION

Kin selection is often invoked to explain the evolution of cooperation among relatives in social animals (Rubenstein and Abbott, 2017). Here, we use ‘social’ to describe species that exhibit cooperative breeding, following the widely used definition of cooperative breeding as a reproductive system in which more than a pair of individuals collectively raise young in a single brood or litter (Emlen and Vehrencamp, 1985; Koenig and Dickinson, 2016). Hamilton’s rule predicts that cooperation confers indirect fitness benefits and will be selected for providing that the coefficient of relatedness between actor and recipient, multiplied by the benefits of cooperation to the recipient exceed the costs to the actor (Hamilton, 1964). Therefore, differential treatment of conspecifics that vary in genetic relatedness, i.e., kin discrimination (Sherman et al., 1997), is an important consideration in studies of social evolution. In addition to kin-selected fitness benefits, kin discrimination may also play an important role in inbreeding avoidance when passive processes, such as sex-biased dispersal, are insufficient to reduce inbreeding risk (Pusey and Wolf, 1996). These functional benefits of discriminating kin from non-kin are well-established, but the mechanisms through which this is realized are keenly debated.

TABLE 1 | Key terms in kin recognition research.

Term	Definition
Kin discrimination	The differential treatment of conspecifics within a population that differ in their genetic relatedness (Sherman et al., 1997)
Kin recognition	The mechanism by which kin discrimination is achieved. A discriminating individual, or *actor*, acquires *cues* to kinship from a *referent* (itself, a subset of kin, or the local environment) and uses these cues to form a *template* (Reeve, 1989). This template is compared with the phenotype of an encountered conspecific, or *recipient*, and an assessment about kinship is made based on the perceived similarity between the template and the recipient's phenotype (Lacy and Sherman, 1983). A specific action is then taken, based on this assessment
Recognition cue	A phenotypic trait expressed by a individual that acts as a reliable signal of kinship, whereby within populations, similarity at the phenotypic trait is correlated with genetic similarity across the genome
Recognition template	An internal representation of kin traits with which the phenotypes of encountered conspecifics can be compared. Templates are usually formed by learning the recognition cues of putative kin, or ones own cues. Templates may also conceivably be genetically determined
Recognition errors	*Desirable* recipients are those which, following acceptance, provide greater fitness pay-offs to the actor than *undesirable* recipients (Reeve, 1989). Within populations, recognition templates are matched against a finite set of cues which overlap in *desirable* and *undesirable* recipients due to individual variation (Lacy and Sherman, 1983). Therefore, any recognition system will involve a certain amount of *acceptance errors*, where undesirable recipients are accepted, and *rejection errors*, where desirable recipients are rejected (Reeve, 1989)

Our current framework for understanding kin recognition systems involves three components: the production of external cues; the perception of these cues and formation of recognition templates; and the action taken based on the perceived similarity between a template and an encountered phenotype (Beecher, 1982; Reeve, 1989; Gamboa et al., 1991; **Table 1**). Both the cue and the template may be either genetically determined or acquired from the biotic or abiotic environment (Sherman et al., 1997). Recognition systems will also be prone to errors; in the case of positive discrimination in favor of kin for helping behavior, these will be either rejection errors, in which kin are not recognized as such and rejected as social partners, or acceptance errors in which non-kin are erroneously recognized as kin and accepted as social partners (Reeve, 1989; **Table 1**). The extent to which cues and templates are determined genetically and/or environmentally, and the risk of making rejection/acceptance errors will vary greatly between and within species (Sherman et al., 1997; Komdeur et al., 2008).

This framework leads to three broad categories of kin recognition mechanism. Recognition may be based on familiarity, in which discriminating individuals learn the recognition cues of relatives (e.g., parents and/or siblings) at a sensitive phase during development (Komdeur and Hatchwell, 1999) and discriminate these familiar individuals from unfamiliar ones later in life. Second, recognition may be based on phenotype matching, whereby individuals use their own phenotype and/or those of their familiar kin to form a generalized template with which to compare the phenotypes of other individuals (Lacy and Sherman, 1983). Familiarity and phenotype-matching are considered alternative processes (Holmes and Sherman, 1983), but both involve matching phenotypes to learned templates; the two mechanisms differ only in the specificity of the template employed (Reeve, 1989). Thirdly, it is also possible that both cues and templates are genetically-determined rather than environmentally-acquired or learned, thereby satisfying Grafen's (1990) definition of kin recognition as requiring discrimination of true genetic relatives, although note that here we use the less restrictive definition of Sherman et al. (1997), as stated above.

The ecological and social circumstances in which a recognition system evolves is likely to have a profound effect on the probable mechanism of recognition (Komdeur et al., 2008). Likewise, a species' kin recognition mechanism will have consequences for the accuracy of discrimination and the degree of resolution between different categories of kin. For example, kin recognition that requires prior association for the learning of cues or templates allows individuals to recognize familiar kin only, whereas recognition that is based on phenotype matching may permit recognition of unfamiliar kin (Mateo, 2004). Among cooperatively breeding birds, recognition of familiar individuals is usually regarded as the most plausible mechanism for kin recognition (Komdeur and Hatchwell, 1999). However, the term familiarity is often ill-defined, the recognition cues are poorly understood, and very little is known about the conditions under which a previous association constitutes familiarity in the context of kin recognition. In this article, we first review the evidence for alternative kin recognition mechanisms in social birds, concluding that recognition based on familiarity is the best-supported decision rule for discrimination of kin from non-kin in most studies. We then consider some of the complexities of familiarity as a cue to kinship, suggesting that while such a mechanism for kin recognition may appear more parsimonious and widespread than phenotype matching, its apparent simplicity is deceptive. Finally, we discuss possible directions for future research on familiarity as a kin recognition mechanism in social birds and other taxa.

MECHANISMS OF KIN RECOGNITION

Kin recognition may be achieved via a variety of mechanisms that range from simple to complex. In the simplest form of recognition, individuals encountered in a particular area are recognized as kin. As long as relatives are predictably distributed in space, location can correlate reliably with genetic relatedness (Komdeur and Hatchwell, 1999). Some researchers suggest this is not a true form of kin recognition, as individuals are responding to location, rather than phenotypic cues (Halpin, 1991; Tang-Martinez, 2001). However, in many natural populations, it is rare for unrelated individuals to be encountered in the nest for example, and a simple decision rule such as "treat anything in my nest as kin," is an effective and widely used mechanism for offspring recognition in birds (Beecher, 1991), despite its

TABLE 2 | Mechanisms of kin recognition.

Mechanism	Definition
Genetic kin recognition	All three components of the recognition system are determined by a specific allele or gene complex (Mateo, 2004). Genetic kin recognition does not require a period of learning in order for templates to form
Phenotype matching	Individuals use their own phenotype and/or those of their familiar kin to form a generalized template with which to compare the phenotypes of other individuals (Greenberg, 1979; Holmes and Sherman, 1982). Because learned templates are generalized, a period of previous association is not required for kin to recognize one another. Instead, a positive correlation between cue similarity and level of genetic relatedness is required, so the recipients with phenotypes that most closely match the actor's general template are its closest kin (Tang-Martinez, 2001)
Familiarity	The recognition cues of putative relatives are learned and used to form templates during a sensitive phase during development (Komdeur and Hatchwell, 1999), within which associating individuals are likely to be kin. Individuals are subsequently able to discriminate these familiar individuals from unfamiliar ones outside of the association period. Recognition based on familiarity therefore requires a period of prior association for individuals to be categorized as kin

potential for exploitation by intra- and inter-specific brood parasites (Davies, 2000). Other contextual cues may modify this simple rule; for example, polyandrous male dunnocks *Prunella modularis* are more likely to feed the young of females with which they mated during their fertile period (Burke et al., 1989; Davies et al., 1992), thereby maximizing their chance of directing their care toward offspring. Spatial cues to offspring recognition may be superseded by individual recognition when fledglings leave the nest (Beecher, 1988), but, in most cases, parent-offspring recognition does not persist beyond the period of offspring dependence.

Such simple rules work well in non-social species, in which there is little or weak selective pressure to recognize kin beyond offspring independence. However, in social species there are often indirect fitness benefits to be gained from cooperating with close kin during adulthood or fitness costs of inbreeding, and, consequently, selection for mechanisms of kin recognition that persist beyond the period of parental care (Komdeur and Hatchwell, 1999; Cornwallis et al., 2009). In this review, we focus on mechanisms in social birds that might permit kin recognition over an individual's lifetime, or at least the period over which cooperative behavior or the risk of inbreeding exists. Such mechanisms may be based on genetic kin recognition, phenotype matching or familiarity (**Table 2**).

Genetic Recognition

Genetic kin recognition requires discrimination of kin from non-kin based entirely on genetically acquired cues without a period of associative learning. Here, *recognition alleles*, dubbed "greenbeard genes" by Dawkins (1976) or gene complexes encode the production of phenotypic cues, the templates and the perception of the cue and performance of a discriminatory action. Such a system relies on polymorphic recognition genes for reliable discrimination, yet paradoxically, kin-selected fitness benefits are predicted to reduce allelic diversity at these loci. This is because in cooperative contexts, individuals bearing common cues are more likely to encounter equivalent individuals and receive altruistic benefits than those with rare cues. These individuals will gain higher fitness, and eventually the common alleles become fixed and the recognition system breaks down (Crozier, 1986). Alternatively, mutation will interfere with genetic kin recognition, and mutant cheats who carry the phenotypic cues but not the associated relatedness, may evolve and spread through the population (Hamilton, 1964). Finally, in the case of a gene complex orchestrating recognition, recombination could disrupt kin recognition. In each of these theoretical scenarios, the required correlation between similarity in the inherited phenotypic cue and kinship among pairs of individuals would decrease over time, rendering such a cue useless for kin recognition (Gardner and West, 2007). There are no convincing cases of genetic kin recognition in cooperatively breeding birds (**Table 3**). Indeed, empirical evidence of genetic kin recognition across taxa is scarce, the clear exceptions being the slime mold, *Dictyostelium discoideum* (Queller et al., 2003) and fire ant *Solenopsis invicta* (Keller and Ross, 1998; Wang et al., 2013).

Phenotype Matching

The second candidate mechanism for kin recognition is phenotype matching. The distinction between phenotype matching and genetic kin recognition is that template formation requires the learning of phenotypic cues that reliably reflect genetic similarity. However, because individuals can use their own phenotype or the phenotypes of a subset of known kin to learn a generalized "kin" template, this does not require a period of prior association, or familiarity between matching individuals. Phenotype matching is an attractive potential mechanism for kin recognition, particularly in the context of inbreeding avoidance, because it allows individuals to recognize unfamiliar kin. Phenotype matching has been demonstrated in the decorated cricket *Gryllodes sigillatus* (Capodeanu-Nägler et al., 2014) and in several social mammals (e.g., Boyse et al., 1991; Pfefferle et al., 2013). Although in some species, such as the Belding's ground squirrel *Spermophilus beldingi* both phenotype-matching and familiarity seem to play a role (Holmes and Sherman, 1982). However, empirical support for phenotype matching in cooperatively breeding birds remains rare and inconclusive (**Table 3**).

One of the first studies to suggest phenotype matching as a plausible kin recognition mechanism in a cooperative bird was conducted by Price (1998, 1999) on stripe-backed wrens *Campylorhynchus nuchalis.* A series of playback experiments demonstrated that wrens were able to discriminate between vocalizations made by their own group, familiar neighboring groups and unfamiliar groups, consistent with a recognition system based on familiarity (Price, 1998). Subsequent experiments showed that the behavioral responses of wrens to calls from patrilineal relatives in the unfamiliar groups did not differ from their responses to calls from patrilineal relatives in their own group, which could indicate phenotype matching

TABLE 3 | Summary of empirical field studies of cooperatively breeding birds in which kin or group discrimination has been identified.

Species	Cue	Origin	Recognition mechanism	Protocol	Evidence	References
Acorn woodpecker *Melanerpes formicivorus*			Familiarity	Field observations	Females do not breed in their natal group as long as their known or presumed father is still present Reproductive vacancies remain unfilled by related nonbreeding helpers of the missing sex	Koenig and Pitelka, 1979; Koenig et al., 1999
Apostlebird *Struthidea cinerea*	Vocal			Call similarity analysis and playback experiments	Individuals can differentiate between different group members by call, but there is no relationship between call similarity and genetic relatedness	Warrington et al., 2014a,b
Arabian babbler *Turdoides squamiceps*	Spatial		Spatial information	Field observations	All nestlings present in the territory are fed at a similar rate, regardless of kinship or genetic similarity	Wright et al., 1999
Bell miner *Manorina melanophrys*	Vocal	Genetic	Phenotype matching	Call similarity analysis	Vocal similarity correlates with genetic similarity and helper effort. The relationship between call similarity and helper effort persists after exclusion of known first-order kin	Wright et al., 2010; McDonald and Wright, 2011; McDonald et al., 2016
Chestnut-crowned babbler *Pomatostomus rifuceps*	Vocal		Familiarity	Playback experiments	Groups react more strongly to the playback of familiar group members than unfamiliar individuals from other groups	Crane et al., 2015
Galápagos mockingbird *Nesomimus parvulus*			Familiarity	Field observations, cross-fostering experiments	More birds help at nests where both breeders fed the potential helper as a nestling than where one or both breeders have not. Nestling swaps do not affect behavior, so preferences are based on the identity of breeders. Helper rates do not vary with relatedness	Curry, 1988
Green woodhoopoe *Phoeniculus purpureus*	Vocal		Familiarity of group members or recognition of a converged group signature	Call similarity analysis, playback experiments	Groups have acoustically distinct rallies. Groups respond differently to rallies of neighbor groups than to stranger groups	Radford, 2005
Long-tailed tit *Aegithalos caudatus*	Vocal	Learned	Familiarity	Call similarity analysis, playback experiments, cross-fostering, field observations	Individuals recognize familiar kin using calls learned during development. Helping and mate choice models show strong discrimination of first order kin but not second order kin	Russell and Hatchwell, 2001; Sharp et al., 2005; Nam et al., 2010; Leedale, 2018; Leedale et al., 2020
Mexican jay *Aphelocoma wollweberi*	Vocal		Familiarity of group members or recognition of a converged group signature	Playback experiments	Jays respond more strongly individuals from other groups than to group members	Hopp et al., 2001
Noisy miner *Manorina melanocephala*	Vocal			Field observations, playback experiments	Related helpers provision offspring more often than unrelated helpers. Noisy miners can differentiate individuals based on vocal cues	McDonald, 2012; Barati et al., 2018
Red-cockaded woodpecker *Leuconotopicus borealis*			Familiarity	Field observations	Female breeders abdicate following the death of their mate when remaining males are sons, but remain when male helpers are unrelated	Walters et al., 1988

(Continued)

TABLE 3 | Continued

Species	Cue	Origin	Recognition mechanism	Protocol	Evidence	References
Seychelles warbler *Acrocephalus sechellensis*			Familiarity	Cross-fostering	Birds become helpers at nests belonging to individuals who fed them as nestlings, even if they are not always the most genetically related. Females are more likely to help than males, and the decision to help is based on whether the breeding female previously fed the helper	Komdeur, 1994; Richardson et al., 2003; Komdeur et al., 2004
Siberian jay *Perisoreus infaustus*			Familiarity and phenotype matching?	Cross-fostering, field observations	Cross-fostering experiments demonstrate equal tolerance toward genetic and foster offspring. Aggression of male breeders toward immigrants is negatively associated with genetic relatedness	Griesser et al., 2015
Splendid fairy-wren *Malurus splendens*	Vocal		Familiarity	Playback experiments	Wrens respond aggressively to songs of wrens from other social groups. Wrens respond similarly to songs of non-kin and unfamiliar close kin	Payne et al., 1988
Stripe-backed wren *Campylorhynchus nuchalis*	Vocal		Familiarity and phenotype matching?	Playback experiments	Wrens discriminate between the calls of unrelated neighboring groups and unfamiliar groups, and they discriminate both of these from calls of their own groups. Responses to calls from presumably unfamiliar patrilineal relatives in other groups do not differ from responses to those in own groups. Calls are likely to be learned during development	Price, 1998, 1999
Superb starling *Lamprotornis superbus*	Vocal		Familiarity	Call similarity analysis, playback experiments	Flight calls are more similar within groups than within the larger population. Call similarity is uncorrelated with genetic relatedness	Keen et al., 2013
Western bluebird *Sialia mexicana*	Vocal		Familiarity	Playback experiments, field experiments	Familiar kin are actively avoided as mates when pairing occurs in winter groups. Males respond more aggressively toward songs of non-kin than songs of kin, but call similarity does not indicate kinship	Açkay et al., 2013, 2014; Dickinson et al., 2016
White-fronted bee eater *Merops bullockoides*			Familiarity	Field observations	Helpers preferentially help kin; the probability of helping decreases with relatedness between helper and potential recipient. Recognition is based on early associations formed during nestling or fledgling development	Emlen and Wrege, 1988

(Price, 1999). However, patrilineal relatives in unfamiliar groups are dominant males that have dispersed from their natal group, so a period of association between the dominant male in each group cannot be ruled out. As male helpers may follow the dominant male in their behavioral responses to intruders, this result could be achieved through recognition based on familiarity.

A recent study on Siberian jays *Perisoreus infaustus*, a species that exhibits kin-based sociality although not cooperative breeding, has suggested that phenotype matching is used to recognize kin in some contexts. Within family groups, breeders are more aggressive toward immigrants than to their own offspring, but aggression of breeders toward immigrants was negatively associated with the immigrant's genetic relatedness to the breeding male (Griesser et al., 2015). In this study, individuals were considered unfamiliar if they had not interacted between fledging and dispersal, although the possibility that individuals had prior association could not be ruled out unequivocally.

Studies of bell miners *Manorina melanophrys* provide the best evidence for kin recognition via phenotype matching in cooperatively breeding birds (McDonald and Wright, 2011). Certain features of the bell miner's social system have important consequences for their recognition systems. They form large colonies, often comprising hundreds of individuals, within which individuals are organized into *coteries* of numerous breeding pairs assisted by non-breeding helpers of varying relatedness that provision multiple nests within their coterie. Like many cooperative breeders, kinship appears to be the most important factor in explaining the patterns of cooperation between breeders and helpers (Wright et al., 2010) and the shared provisioning efforts of helpers within social networks (McDonald et al., 2016). From an early age, however, young interact with both related and unrelated group members, making spatial or association-based recognition unreliable. Instead, the provisioning effort of helpers correlates with their vocal similarity to the breeding male, an apparently innate signal that also correlates with genetic relatedness (McDonald and Wright, 2011). However, whether vocal similarity permits kin recognition on a continuous scale or on a binary scale, whereby conspecifics are categorized as either kin or non-kin based on a threshold of template-phenotype similarity, remains unclear. Furthermore, although no evidence of call learning has been found, a putative association period during which kin may be learned has not been excluded empirically.

In the closely related noisy miner *Manorina melanocephala*, which has a similar social system, helpers direct their help toward genetic relatives (Barati et al., 2018), and discriminate between individuals based on acoustic cues (McDonald, 2012). Still, individuals may also rely on prior association to identify relatives, and whether kin recognition is based on phenotype matching or familiarity remains untested in this species.

The problem with recognition via phenotype matching of inherited cues is that, like genetic kin recognition, it is vulnerable to mutation and recombination, and requires sufficient polymorphism to permit precise discrimination. Another important consideration is that there may be selection for individuals to conceal kinship at certain life stages or in certain situations. For example, when paternity is uncertain, effective kin recognition by parents would be adaptive in order to direct care toward genetic offspring. However, from the offsprings' perspective, it would not be beneficial to display an obvious cue to genetic relatedness, as this could exclude cuckolded care-givers (Beecher, 1988; Davies et al., 1992). This conflict of interest between parent and offspring may make it difficult for phenotype matching of genetic cues to evolve as a recognition mechanism. Even if recognition cues are learned, the formation of a generalized template may still select for convergence, as individuals with a more common phenotype are more likely to be accepted as social partners than those with rarer cues. On the other hand, theory suggests that genetic diversity at recognition loci may be maintained if rare alleles confer an extrinsic selective advantage, such as resistance to certain parasites (Rousset and Roze, 2007). Indeed, the highly polymorphic major histocompatibility complex (MHC), has been implicated as a kinship marker during mate choice in vertebrates, detected through odor cues. MHC diversity affects parasite resistance (Kurtz et al., 2004), perhaps explaining how MHC polymorphism is maintained despite its putative role in kin recognition. However, the role of MHC in kin recognition is contested, as disassortative mate preference based on MHC haplotype may arise from the improved immunity associated with heterozygosity at MHC loci itself, rather than MHC haplotype acting as a reliable signal of genetic similarity across the genome (Green et al., 2015).

Familiarity

Familiarity is the most widely supported mechanism of kin recognition in cooperatively breeding birds (Komdeur and Hatchwell, 1999; Komdeur et al., 2008; Riehl and Stern, 2015; **Table 3**). Kin association during extended brood care provides a sensitive period during which reliable recognition templates can form. This period of association also offers an opportunity for learning of cues that are more similar within a family than in the general population, termed a family or kin "signature" (Beecher, 1982). Once recognition cues are fixed, individuals are potentially able to recognize familiar kin outside of the association context. When extra-pair paternity (EPP) and brood parasitism is rare, association during this period accurately reflects kinship, and a simple rule such as "assist anyone who was present in my natal nest" can be selected for (Komdeur and Hatchwell, 1999). For example, in cooperative contexts, Galápagos mockingbirds *Nesomimus parvulus* and white-fronted bee-eaters *Merops bullockoides* discriminate based on previous association, rather than kinship (Curry, 1988; Emlen and Wrege, 1988). In complex societies, a more precise rule, such as "assist anyone that fed me as a nestling" may be more reliable (Komdeur, 1994). In most cooperatively breeding birds, males are the predominant helping sex, but in the Seychelles warbler *Acrocephalus sechellensis* females are more likely to help than males, and choose to help at nests belonging to female breeders who fed them as nestlings, even if they are not the closest genetic relatives (Komdeur, 1994; Richardson et al., 2003). This makes evolutionary sense in species with high levels of extra-pair paternity, such as Seychelles warblers, because helpers are often unrelated to the male that fed them (Richardson et al., 2003).

Cross-fostering experiments confirm that female subordinates base their helping decisions on associative learning and it is unlikely that young can discriminate between their mother and any other female helper (Komdeur et al., 2004).

Playback experiments show that cues enabling recognition of familiar individuals beyond the association period are encoded vocally (**Table 3**). An early study on the splendid fairy-wren *Malurus splendens* showed that while fairy-wrens responded aggressively to the songs of fairy-wrens from other social groups, they exhibited a similar response to the songs of both non-kin and unfamiliar close kin (Payne et al., 1988). More recent experiments have demonstrated that vocalizations signal group membership in Mexican jays *Aphelocoma wollweberi* (Hopp et al., 2001), green woodhoopoes *Phoeniculus purpureus* (Radford, 2005) and superb starlings *Lamprotornis superbus* (Keen et al., 2013). These studies suggest that vocalizations reflect social association rather than kinship *per se*, as would be expected if cues and templates are learned within groups.

In the context of inbreeding avoidance, good evidence for avoidance of kin as reproductive partners based on familiarity comes from studies of two species of social woodpecker: acorn woodpeckers *Melanerpes formicivorus* and red-cockaded woodpeckers *Picoides borealis*. Acorn woodpeckers exhibit high within-group relatedness, with most individuals being parents, siblings or offspring of everyone else within the group (Koenig and Haydock, 2004). Acorn woodpecker females do not breed in their natal group when the reproductive male in their natal group at the time of their birth (their assumed father) is still present (Koenig and Pitelka, 1979). Furthermore, when a dominant male or female dies, reproductive vacancies remain unfilled when non-breeding helpers of the missing sex are present, and breeding does not usually occur until the vacancy is filled by immigrants from outside the group (Koenig et al., 1999). Similarly, red-cockaded woodpecker females will abdicate a breeding position following the death of their mate when the remaining males are their sons, but will remain when they are unrelated to the male helpers (Walters et al., 1988). The mechanism behind these decisions has not been examined experimentally in either species.

The most compelling cases of kin recognition based on familiarity come from cooperative breeders in which helping occurs within kin neighborhoods (Dickinson and Hatchwell, 2004), where individuals routinely interact socially with both kin and non-kin so that selection for effective kin discrimination is likely to be strong (Cornwallis et al., 2009). In western bluebirds *Sialia mexicana* there is a strong kin preference in helping behavior (Dickinson et al., 1996) and active kin avoidance during mate choice (Dickinson et al., 2016). However, males do not reduce their provisioning effort in response to behavioral cues to paternity loss, such as extra-pair male intrusion or witnessing female acceptance of extra-pair copulations (Dickinson, 2003). This suggests, along with earlier studies (Leonard et al., 1995), that males do not recognize their own offspring, and that discrimination by both parents and offspring is based on social experience in the nest, rather than genetic relatedness (Dickinson, 2003). Playback experiments have shown that individuals discriminate kin based on vocal cues (Açkay et al., 2013) even though these vocalizations are poor indicators of genetic relatedness, because they are most similar among neighbors, regardless of kinship (Açkay et al., 2014). These findings collectively suggest that western bluebirds recognize familiar individuals, rather than kin, using vocal cues.

Kin recognition has also been extensively studied in another species that helps within kin neighborhoods, the long-tailed tit *Aegithalos caudatus*. Long-tailed tits have a kin-selected cooperative breeding system in which failed breeders preferentially redirect their care to help relatives (Russell and Hatchwell, 2001; Hatchwell et al., 2014). Playback experiments show that long-tailed tits are able to discriminate between the calls of close kin and non-kin (Hatchwell et al., 2001; Sharp et al., 2005), and the calls thought to be used as recognition cues are individually distinctive, repeatable and more similar among close kin than among non-kin (Sharp and Hatchwell, 2005; Leedale et al., 2020). Cross-fostering experiments showed that nestlings and/or fledglings acquire their recognition templates from familiar kin during an associative learning period, when the cues themselves develop (Sharp et al., 2005), and that cross-fostered offspring subsequently help at the nest of foster siblings (Hatchwell et al., 2001). Moreover, there is strong evidence for effective discrimination of first-order kin, but not second-order kin, both in the context of helping behavior and mate choice (Leedale, 2018; Leedale et al., 2020). These results are all consistent with the idea that long-tailed tits categorize conspecifics as either kin or non-kin based on early association in the context of brood care (Sharp et al., 2005). On the other hand, Nam et al. (2010) and Leedale et al. (2020) both found that long-tailed tit helpers modified their effort according to their relatedness to the helped brood, suggesting that assessment of kinship is not based on a simple dichotomous rule of familiar (kin) vs. unfamiliar (non-kin) birds. Indeed, this suggests a mechanism of phenotype matching, with a gradation of similarity in vocalizations providing a fine-grained, continuous estimation of kinship. However, bioacoustic analysis did not support this suggestion (Leedale et al., 2020), so even in this relatively well-studied system, the mechanism underlying graded discrimination remains unknown.

This review focuses on kin recognition, but familiarity also provides a potential mechanism by which individual recognition may be achieved; for example, some cooperative bird species, such as the chestnut-crowned babbler *Pomatostomus ruficeps* have individually distinct vocalizations (Crane et al., 2015). However, although individual recognition has been identified in several social mammals, including chacma baboons *Papio hamadryas* (Bergman, 2003) and golden hamsters *Mesocricetus auratus* (Johnston and Bullock, 2001), there are no conclusive examples of individual recognition in cooperatively breeding birds (**Table 3**). The difference between individual and group recognition depends on the specificity of the templates acquired during the association period, which in turn depends on the nature of the interactions that occur between individuals during that time. In practice, this makes distinguishing individual from kin or group recognition difficult (Tibbetts and Dale, 2007). We discuss this in more detail in the following section.

Overall, there is substantial evidence that familiarity is a widespread kin recognition mechanism in cooperatively breeding birds. The limitation of familiarity is that non-kin will be considered kin if they are encountered during the putative associative learning stage, and kin not encountered during this period will not be recognized as such. However, in most cases, proximity at certain life stages is a reliable indicator of kinship. This is particularly true of birds, which have a prolonged period of parental care at the nest where encountered individuals are likely to be close kin. A second assumed limitation of recognition based on familiarity is that it may result in a binary recognition rule, in which individuals are categorized as either kin or non-kin. A more sophisticated mechanism that permits relatedness to be assessed on a continuous scale would be adaptive, in accordance with Hamilton's rule (Hamilton, 1964), although, as already discussed, such mechanisms may be evolutionarily unstable. Kin recognition through familiarity or prior association is also considered the most likely mechanism of kin recognition in social birds because it is simpler to evolve and arguably less cognitively demanding than an assessment of genetic relatedness based on phenotypic similarity. Yet, while a recognition system based on familiarity may be more parsimonious and widespread than phenotype matching and genetic mechanisms, we argue below that its apparent simplicity is deceptive.

THE COMPLEXITIES OF FAMILIARITY AS A CUE TO KINSHIP

Despite the general acceptance of familiarity as an important means of kin recognition and discrimination, much remains unknown about how associating individuals are categorized as kin and how familiar individuals are recognized after the associative learning period. Here, we suggest that progress will be made in understanding familiarity as a mechanism of kin recognition only when certain gaps in knowledge can be addressed: (i) the meaning of "familiarity," (ii) the sensitive period for association; (iii) the cues used for recognition; and (iv) the distinction between familiarity and phenotype matching.

What Is "Familiarity"?

Familiarity in the context of kin recognition is difficult to define and to quantify. What is the specific series of events during which an individual learns who is familiar? In the kin recognition literature, familiarity generally refers to some previous social association among individuals, usually during early life stages (Hepper, 1986; Komdeur and Hatchwell, 1999), but the nature of this association is often vague. For instance, is spatial proximity sufficient, or do individuals need to interact in specific ways in order to become familiar? In studies of social birds, such as long-tailed tits, spatial proximity of nestlings may provide the basis for future helping among siblings, but helping also occurs across generations indicating that association when provisioning a brood or when being provisioned also provides the basis for future helping (Sharp et al., 2005; Nam et al., 2010). Precisely when the interactions took place, how many interactions there were, their duration, and the specific behavior and information transfer that took place during these interactions may influence how individuals are recognized and treated later in life. A critical issue here is that individuals often become familiar with and recognize many conspecifics through their lifetime, including mates (Blumenrath et al., 2007), territorial neighbors (Stoddard, 1996) or flock mates (Nowicki, 1983), so is it the timing, frequency or nature of the social interaction that results in some individuals being treated as kin and others not? A particularly nice example of such context-specificity in kin recognition is suggested by Komdeur et al.'s (2004) finding that Seychelles warbler helpers assist in the rearing of half-siblings that are the offspring of their mother but not those of their father, even though both parents would have provisioned the helper when it was young.

It may also be possible for individuals to acquire cues to kinship based on observations of the behavior of their familiar relatives toward other individuals. For example, unfamiliar individuals observed engaging in positive interactions with one's parents could be treated as kin. Indeed, such "indirect familiarity" could provide a kin recognition mechanism through which individuals recognize their younger siblings, despite not being reared together. Although we are not aware of any evidence for indirect familiarity among cooperative breeders, this idea parallels the social interaction expected under indirect reciprocity, in which help is directed toward an individual who has been observed providing help to others (Nowak and Sigmund, 2005). However, indirect cues to kinship are likely to be more error-prone than those learned through direct association because the link between kinship and familiarity will tend to be diluted. For example, in the case of direct association among parents, offspring and siblings during rearing, kinship of familiar individuals will usually be consistently high. But, if an offspring observes their parent interacting positively with an uncle, say, its relatedness to the "indirectly familiar" individual is lower than that between directly familiar individuals. If the offspring subsequently helps its uncle, and this is observed by their offspring, the relatedness between such "indirectly familiar" individuals is further reduced. As with direct familiarity, the frequency and nature of the interactions observed must also be considered, which, overall, may make the behavior of others a noisy and unstable cue to kinship.

Social network analysis is being used increasingly to quantify the strength of association between individuals and can be applied at different life history stages (Kurvers et al., 2013; McDonald et al., 2016). A social network inevitably reflects the nature of the behavior used to construct it (Madden et al., 2012), and they do not necessarily reflect genetic relatedness alone (Godfrey et al., 2014). For example, Napper and Hatchwell (2016) found that helping decisions in long-tailed tits reflected not only kinship, but also individuals' spatial distribution and their social associations during the previous winter. More work is needed to evaluate how prior association affects kin-directed behaviors using precisely quantified social networks in different contexts and life history stages.

When Is the Sensitive Period?

There is good evidence that kin recognition requires a period of learning, but when is this critical period? Many vocal learners have a sensory learning phase or window when they learn songs that they sing during adulthood (Kroodsma, 1978). Once this window closes, most songbirds are unable to learn new songs, although their repertoire may later be modified in some species (Mooney et al., 2008). Studies of songbirds show that that the window can be very short with a long delay between the sensory learning phase and the sensorimotor phase, during which the song is rehearsed and perfected, e.g., swamp sparrows *Melospiza georgiana* (Marler and Peters, 1982). Likewise, offspring that imprint on parents have a sensitive imprinting period (Bateson, 1964), and it has been suggested that learning of parental calls may even precede hatching, resulting in a parent-specific password, in superb fairy-wrens *Malurus cyaneus* (Colombelli-Négrel et al., 2012). This is interpreted as defense against inter-specific brood parasitism, but selection for early parent-offspring recognition would also be expected whenever there is a substantial risk of mis-directed parental care. For example, parents in colonial bank swallows *Riparia riparia* accept any offspring in their nest before 15 days, then recognize their own offspring at 15–17 days, i.e., just before fledging (Beecher, 1982, 1988, 1991).

Based on these parallels between bird song learning and associative learning of kin, we postulate that the critical period for learning the template for recognition of kin through familiarity is similar to the sensory learning phase in many vocal learners. Thus, individuals could discriminate kin from non-kin even though they also associate with non-kin before they start vocalizing (Radford, 2005) or cooperating, and any associations that occur after the sensory learning phase (but before the sensorimotor phase) might result in non-kin being disregarded as social partners (i.e., associated but not "familiar"). Cross-fostering experiments provide strong empirical support for this putative learning period (Hatchwell et al., 2001; Komdeur et al., 2004; Sharp et al., 2005). However, although the time of call development is known in some species (e.g., Sharp et al., 2005), the precise timing of kin recognition template formation has not been identified in any cooperatively breeding species. Furthermore, while this mechanism may be effective as a rule for reliably directing care toward kin when mature offspring help their parents or siblings to raise subsequent broods, as is typical of many cooperatively breeding birds (Cockburn et al., 2017), there are species in which helpers care for the offspring of a younger generation of breeders (e.g., Richardson et al., 2007; Nam et al., 2010), suggesting that older birds can learn the identity of younger relatives, a process that must occur outside the putative critical learning period. A similar conclusion must be drawn when parents avoid breeding with younger relatives, as in acorn woodpeckers (Koenig et al., 1999).

Therefore, while the parallels with song-learning are intuitive and appealing, there are clearly situations in which a single sensitive period for learning kin identity do not apply. Cross-fostering experiments targeted at different life history stages and social network analysis across lifetimes provide invaluable tools with which to address this problem, but there remain formidable challenges to achieving a better understanding of the putative learning phase in natural populations.

What Are the Recognition Cues?

Another challenge when determining the role of familiarity is determining the cues used in kin recognition. Vocal cues are the most likely mechanism in birds (**Table 3**), but this has been the default sensory modality in all of the cited studies, so visual and olfactory cues cannot be ruled out. Kin recognition mechanisms in several non-cooperatively breeding birds, particularly in the context of inbreeding avoidance, have focused on odor cues (Coffin et al., 2011; Krause et al., 2012). Storm petrels *Hydrobates pelagicus* prefer non-kin odors when choosing mates (Bonadonna and Sanz-Aguilar, 2012) and odor has also been suggested as a recognition cue in zebra finches *Taenopygia guttata* (Caspers et al., 2013, but see Ihle and Forstmeier, 2013). These studies should encourage future work on olfactory kin recognition in cooperatively breeding birds for two reasons. First, most recent evidence of odor-based kin recognition comes from species with enclosed nests, which may retain odor more readily than open nests, thereby promoting the learning and familiarization of nest odors. Many cooperative breeders nest in domed nests or cavities (Price and Griffith, 2017), suggesting that olfactory cues to kinship are plausible. Second, most species in which odor-based kin recognition has been identified live in flocks or breed in colonies, even though they do not breed cooperatively, suggesting that there might be common selection pressures for odor-based kin recognition to evolve. Interestingly, the finding that preen gland secretion chemicals are positively correlated with MHC relatedness in black-legged kittiwakes *Rissa tridactyla* (Leclaire et al., 2014) suggests that phenotype-matching of odor cues is a feasible recognition mechanism, just as in mammals (Green et al., 2015). However, it should also be noted that even less is known about the timing of development, individuality and repeatability of odor profiles than is known about vocal cues.

For any kin recognition cue, whether vocal or odor, to be effective it must carry either an individual or family signature and be individually repeatable from its initial development to the time of discrimination; the same logic applies to a recognition template. Signal convergence therefore presents a significant problem for the stability of any recognition system. Frequent interactions may lead to an increase in phenotypic similarity among individuals. Vocal convergence can be adaptive for coordinated foraging (Bradbury and Balsby, 2016), particularly when birds forage in annual winter flocks that disband each spring. For example, black-capped chickadees *Parus atricapillus*, exhibit vocal plasticity throughout adulthood and vocal convergence can occur within a week of winter flock formation (Nowicki, 1989). However, such species do not breed cooperatively and individuals do not gain indirect fitness benefits from associating with kin. In kin-selected systems, kin recognition cues must be fixed during early development and cannot be updated during adulthood, even when interactions with non-kin are frequent (Radford, 2005; Keen et al., 2013). In long-tailed tits, vocalizations do not change significantly over an individual's lifetime once learned (Sharp and Hatchwell, 2005), but more studies that investigate the plasticity of putative recognition cues are needed. In addition, while the idea of a *signature system*, a specific profile of phenotypic components that vary in their combination from individual to individual, is well-established (Beecher, 1982), most studies continue to focus on a single recognition modality, rather than recognizing that familiarity is likely to be based on a combination of cues, which may minimize convergence and maintain recognition cue diversity and integrity.

Familiarity vs. Phenotype Matching

Although in principle the mechanisms of familiarity and phenotype matching are readily distinguished, in practice this may often not be the case. The two mechanisms differ in the predictions they make about whether the ability to discriminate requires prior association and about the resolution of discrimination. First, familiarity is explicitly dependent on social partners having prior knowledge of each other, whereas phenotype-matching allows recognition of unfamiliar kin. In practice, it is extremely difficult to rule out prior association in most field studies, even in cross-fostering experiments where there is often a period of association between parents and offspring prior to separation (e.g., Hatchwell et al., 2001). Kin recognition cues may even develop during gestation (e.g., Hepper, 1987) or incubation (e.g., Colombelli-Négrel et al., 2012; Dowling et al., 2016). Secondly, familiarity is generally assumed to result in dichotomous classification of conspecifics into familiar (kin) and unfamiliar (non-kin) individuals, while cue-template similarity under phenotype-matching is assumed to be continuous. However, if the recognition system involves a threshold for acceptance/rejection of social partners (Reeve, 1989), then discrimination based on phenotype-matching and familiarity may appear very similar in practice. Equally, it is possible that familiarity could be assessed as a continuous trait, with conspecifics discriminated according to their degree of familiarity.

Thus, the extent to which recognition cues permit kinship to be perceived on a continuous or binary scale is an important aspect of the kin recognition mechanism. When group membership is used to categorize relatives, as in Arabian babblers *Turdoides squamiceps* (Wright et al., 1999), kin discrimination is binary. When recognition is based on phenotype, e.g., white-fronted bee eaters *Merops bullockoides* (Emlen and Wrege, 1988), it may be binary or continuous, depending on the algorithm used to assess kinship. Binary or threshold kin discrimination will be effective in most cooperative breeders living on stable territories that, at least with regard to the helping sex, are mostly made up of first-order relatives, facilitating a decision rule based on prior association (Curry, 1988; Payne et al., 1988; Komdeur et al., 2004). In contrast, a recognition cue that permits individuals to discriminate kin varying in relatedness has been identified only in the bell miner (Wright et al., 2010), even though such fine-scale discrimination has been reported in at least one other species (Nam et al., 2010; Leedale et al., 2020).

The ability of helpers to assess the relatedness of conspecifics continuously may have been overlooked in some cases because of the way in which cooperative behavior is measured. For example, some studies focus on the probability of helping (Curry, 1988; Creel et al., 1991; Dickinson et al., 1996), whereas others measure the amount of help given (Dunn et al., 1995; Wright et al., 1999; Clutton-Brock et al., 2001), and both have been measured in just a few (Emlen and Wrege, 1988; Komdeur, 1994; Russell and Hatchwell, 2001; Nam et al., 2010). Moreover, consideration must also be given to how relatedness is assessed by helpers, especially the possibility of error and degree of resolution achievable (Leedale et al., 2020). These problems pose formidable challenges to empiricists, with more sophisticated observations and experiments required to determine how relatedness is perceived.

CONCLUSIONS

Familiarity is an intuitively plausible mechanism of kin recognition in social birds that, at first sight, appears more parsimonious than alternatives. However, we think that this apparent parsimony is deceptive, so that although most empirical studies support familiarity as the most likely mechanism, we argue that there is a great deal we do not understand about this process. In particular, we have identified four specific issues that would benefit from further investigation, although in making these recommendations, we acknowledge the difficulty of addressing them in natural populations.

AUTHOR CONTRIBUTIONS

AL collated research and drafted the manuscript with BH. AL, BH, and JL contributed to the final version of the manuscript.

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