Natural Variations in Social Behaviors: Phenotypic Consequences and Genetic Differentiation in Paper Wasps

by

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ABSTRACT

Understanding how phenotypes and genotypes respond to changing environments is a central topic in evolutionary biology. Animal behavior may respond rapidly to changing environments, but little is known about the impact these behavioral responses have on the population phenotypes and genotypes. Animal recognition is an ideal system to study intraspecific variation in behaviors because signalers and receivers interact through correlated phenotypes, which allows us to test how phenotypes and genotypes interact. However, the natural intraspecific variation in recognition systems has not been deeply investigated for many types of signals. Determining the natural phenotypic and genetic variation associated with recognition systems is important because then we can better understand the evolutionary and ecological mechanisms that make recognition possible in animals. In chapter one and two, we explored the geographic variation in individual recognition in *Polistes fuscatus* wasps across six populations, from Pennsylvania to Michigan, USA. For individual recognition to occur in *P. fuscatus*, signalers must have a high diversity of within-population facial and abdominal color marks. In addition, receivers must produce a unique behavioral response toward the signaler. For signalers, we measured the within-population diversity of the facial and abdominal marks. For receivers, we experimentally tested whether receivers learn and remember the individual identities of conspecifics. We found that diversity in signaler facial and abdominal marks is different across the studied populations; some populations have a much higher diversity of marks than other populations. Receivers also show geographical variation in the ability to recognize individual identities. Two out of the six studied populations showed the ability to recognize individual

identities. These results suggest that individual recognition is not a species-typical trait; rather, it is flexible and varies across populations of the same species. In chapter two, we explored the genetic structure of the six populations of *P. fuscatus* from chapter 1. For this analysis, we used a genome reduced-representation approach (ddRAD-seq). First, we compared the genetic structure of the six populations. Fst values suggest that there is gene flow between the populations, and Mantel test was not positive for isolation by distance. Second, we performed a GWAS to look for candidate loci under selection given the variation of individual recognition across the populations. BayeScan approach did not find signatures of natural selection on variable loci. These results suggest that selection signatures in the genome associated with individual recognition were not detected by our approach. In chapter three, we studied how social context affects the integration of complex signals in *P. fuscatus*. We experimentally manipulated visual and chemical signals in workers of *P. fuscatus* to determine what signal nest-members use when performing nestmate recognition. We found that workers of *P. fuscatus* use chemical signals (cuticular hydrocarbons) but not visual signals (face and abdominal color marks) for nestmate recognition. Previous work has shown that visual signals used during social interactions mediate dominant behaviors on nests. These data suggest that *P. fuscatus* integrate information from multimodal signals according to certain social requirements. In conclusion, we provide empirical evidence for intraspecific variation in individual recognition. Our results suggest that gene flow between populations can generate scenarios with high phenotypic variation but low genetic differentiation between the populations. Intraspecific variation in behavior might be an underappreciated factor impacting the phenotypic and genetic diversity in social animals.

CHAPTER ONE

INTRODUCTION

Animal social behavior depends on recognition. There are many types of recognition, including self, kin, mate, gender, friend, offspring, predator, and prey recognition. All recognition involves (i) cue production by the signaler, (ii) cue perception and template matching by the receiver, and (iii) a behavioral response by the receiver (Sherman et al. 1997). In this dissertation, we aim to contribute to the understanding of the evolution and maintenance of recognition systems, specifically individual recognition and class-level recognition in the eusocial paper wasp *Polistes fuscatus*. In chapter one we show how intraspecific geographic variation in individual recognition affects the phenotypes in signalers and receivers of *P. fuscatus*. In chapter two, we explore the genetic basis of individual recognition in *P. fuscatus*. In chapter three we study not how recognition systems change across a geographic transect, but how recognition systems are affected by changes in *P. fuscatus'* social context.

In this introduction, we will explain the main characteristics of individual recognition, variations of recognition intraspecifically and across contexts, the cognitive and sensory adaptations in receivers to facilitate accurate recognition, the effects of individual identity signaling on phenotypes and genotypes, and finally hypotheses explaining complex signals integration in animals. This will lay a conceptual base for the following chapters.

Individual recognition

Multiple definitions of individual recognition have been proposed that differ slightly in their specificity and complexity. The most common definition of individual recognition proposes that receivers discriminate a signaler from others based on the signaler's unique characteristics and associate the unique characteristics with individual-specific information about the signaler (Tibbetts and Dale, 2007) (Fig. 1.1). For example, King penguins (*Aptenodytes patagonicus*) exhibit individual recognition because each chick has individual-specific calls (signaler's cue). Parents learn their chicks' unique calls and respond in a unique way; they treat their own chick differently than other chicks (Aubin and Jouventin, 1998).

Individual recognition is interesting from a cognitive perspective because it is considered a relatively specific and complex form of recognition. During individual recognition, receivers must learn the unique features of conspecifics, associate the features with individual-specific information, and recall the feature/information link during subsequent interactions. Individual recognition is also interesting from a cognitive perspective because it allows the formation of individually differentiated social relationships. The potential for individually differentiated social relationships influences the type and sophistication of social interactions possible within a society (Platt, Seyfarth, and Cheney, 2016).

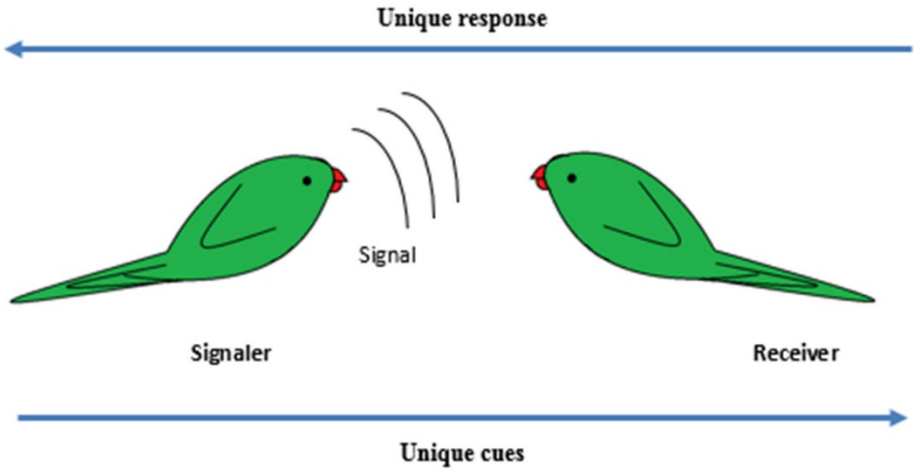

Figure 1.1. The criteria for individual recognition. Signalers have unique cues and receivers behave in a unique way toward the signaler.

Social contexts in which individual recognition is used

Interspecific variation in individual recognition across contexts

Individual recognition is common in social contexts when there are repeated interactions between the same target individuals and also non-target individuals. Such situations include territoriality, dominance interactions, cooperation, providing parental care, and pair-bonding (Tibbetts and Dale, 2007).

Much of the earliest research on individual recognition examined recognition of territorial neighbors (originally termed 'dear enemy' recognition). For example, many birds use song to identify the identity and position of their neighbors. Birds largely ignore playbacks of neighbor songs positioned on the correct territorial boundary, but respond aggressively to playbacks of neighbor songs positioned on the incorrect territorial boundary and to playbacks of stranger songs (Stoddard, 1996). Individual recognition is thought to reduce the energetic costs

of territorial defense by enabling territory holders to focus their aggressive efforts on non-territorial floaters instead of their less-threatening neighbors (Fig. 1.2).

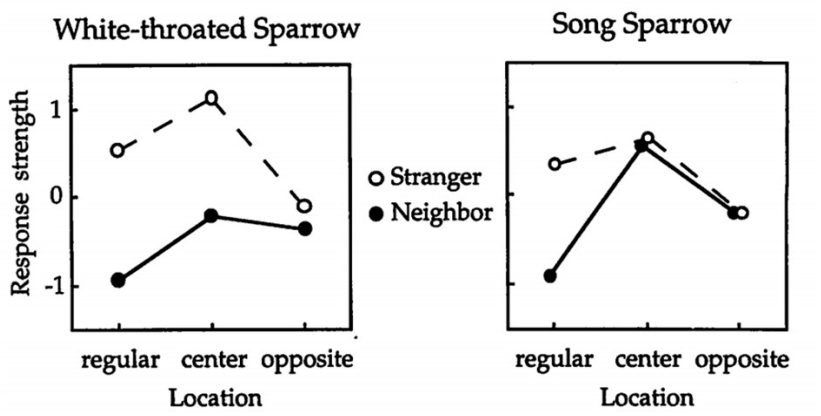

Figure 1.2. The dear enemy approach to testing individual recognition. White-throated sparrow (*Zonotrichia albicollis*) are less aggressive to neighbor songs on the correct territorial boundary than neighbor songs on the incorrect territorial boundary, indicating that White-throated sparrow recall the individual identity and position of neighbors. Speakers played back neighbor and stranger songs from the boundary shared with the neighbor (regular), the center of the territory (center), and the boundary shared with a different neighbor (opposite). The behavioral data are normalized composite scores for the number of flights and the closest approach to the playback speaker. Taken from Stoddard (1996).

Individual recognition is also important during aggressive competition because it can reduce the costs associated with competition and stabilize dominance hierarchies (Barnard and Burk, 1979). Individual recognition is likely to be beneficial when there are repeated aggressive interactions between small numbers of individuals. In this situation, learning the individual identity and competitive ability of conspecifics will provide a precise way to assess rivals without multiple fights. For example, some species of fish, including brook trout (*Salvelinus fontinalis*), individually identify rivals via scent. Trout assess relative dominance ranks based on the outcome of their own aggressive interactions as well as observations of others via transitive inference. If a fish beats rival A in a fight, then observes A win in a contest with B, the fish will correctly infer that it is more dominant than individual B (White and Gowan, 2013). Transitive inference was once thought to be a cognitively complex task restricted to few taxa, but has now been found in many vertebrate species with individual recognition that form dominance

hierarchies (Paz-y-Miño, Bond, Kamil, and Balda, 2004). Individual recognition associated with aggressive competition is widespread in nature, as many species use individual recognition to minimize the costs of conflict (Barnard and Burk, 1979).

Although cooperation can occur without individual recognition, there is much evidence that cooperative behaviors such as reciprocity, alliances, social prestige, punishment of non-cooperators, and image scoring are facilitated by individual recognition. As a result, many taxa with complex social interactions rely on individual recognition. Individual recognition of group members is ubiquitous in primates and also occurs in many species that live in cooperative groups, including mammals, fish, and birds (Sharpe, Hill, and Cherry, 2013). Although social insects have complex social behavior, individual recognition is relatively rare in social insects. Instead, most social insect societies rely on class-level recognition and self-organization to produce apparently complex behavior with little individual cognitive investment.

Individual recognition is involved in multiple aspects of reproduction, including parent-offspring interactions and monogamous pair-bonding. Individual recognition during parental care is particularly likely to occur when there is potential for confusion among offspring, e.g. organisms that breed in high-density groups with synchronous reproduction, and reduced recognition potential based on positional information. For example, the Australian sea lion *Neophoca cinerea*, reproduces in large colonies. Both mothers and pups use individual-specific calls to find each other after the mother's foraging trips (Charrier, Pitcher, and Harcourt, 2009). Monogamous species with biparental care use individual recognition to maintain pair bonds, mate-guard, and defend territories. For example, *Chaetodon multicinctu* butterfly fish use visual and olfactory cues to discriminate their mate from non-mates (Boyle and Tricas, 2014).

Individual recognition during monogamous pairing and parent-offspring recognition are sometimes considered simple forms of individual recognition because receivers need only learn and recall one individual. Some argue 'true' individual recognition requires receivers who can individually discriminate many individuals (Thom and Hurst, 2004). As a result, future work that tests whether multiple mates, parents, or offspring can be individually identified will be useful. For example, in taxa with multiple offspring, do parents treat different offspring uniquely? For instance, there is evidence that parents of the songbird black redstart (*Phoenicurus ochruros*) respond more to the calls of the fledglings that they preferentially fed, suggesting that parents can discriminate between offspring (Draganoiu, Nagle, Musseau, and Kreutzer, 2006).

While most examples of individual recognition occur within species, individual recognition is not restricted to conspecifics. Some animals recognize individuals of other species. For example, mockingbirds learn to assess the level of threat posed by different humans. When the same human approaches a mockingbird's nest for four successive days, mockingbirds begin flushing from the nest when the human is a great distance from the nest and also increase alarm calling and attack flights. Mockingbird response to humans who have not threatened the nest does not change (Levey et al., 2009).

Intraspecific variation in individual recognition across contexts

A large body of research has examined the types of social behavior associated with interspecific variation in individual recognition, but less is known about intraspecific variation in individual recognition. Examining intraspecific variation is important because responses to individual identity signals may vary across the biotic and abiotic environment. For example, a species may use individual recognition during aggressive encounters in certain social or environmental circumstances, but not other circumstances.

Previous work on has shown there is some intraspecific variation in receiver responses to signals. For example, receiver responses to quality signals often vary with traits like sex, maturity, season, habitat, and social context. Many studies have shown that the biotic and abiotic environment influence whether females choose male partners based on sexual signals or ignore sexual signals during partner choice (Searcy and Nowicki, 2005). Such variation occurs because assessing and responding to signals can be costly, so receivers will only pay attention to signals when signal response is sufficiently beneficial. Therefore, intraspecific variation in recognition may occur whenever the benefits of recognition differ across contexts.

A few studies have found intraspecific variation in individual recognition. Notably, pair-bonded male prairie voles learn to recognize individual females, while single males do not learn to recognize females. The authors found no difference in male motivation to contact females across paired and unpaired males, so they speculate that recognition differs because the social relevance of information about females varies across contexts (Blocker and Ophir, 2015).

In the paper wasp *Polistes fuscatus*, the social benefits of individual recognition and receiver capacity for individual recognition vary across castes. Specifically, workers are less able to learn and remember individual conspecifics than foundresses (Tibbetts *et al.* in review). During social interactions, workers are unable to remember unique individuals following a six-day separation, though foundresses remember other foundresses for six days. The differences in individual recognition across castes are initially surprising because *Polistes* lack discrete queen/worker castes. Workers and queens are morphologically similar and workers can take over nests as queens. Difference in recognition may occur because the social benefits of individual recognition differ across foundresses and workers. Workers do not benefit by being individually recognizable on stable queenright nests because worker-worker aggression on queenright nests is

low and workers do not reproduce or have unique roles in the colony. In contrast, foundresses benefit by being individually recognizable, as it reduces aggression and stabilizes social interactions among cooperating foundresses (Fig 1.3). Individual recognition also allows foundresses to keep track of individual social relationships, including division of aggression, food, and work (Sheehan and Tibbetts, 2009).

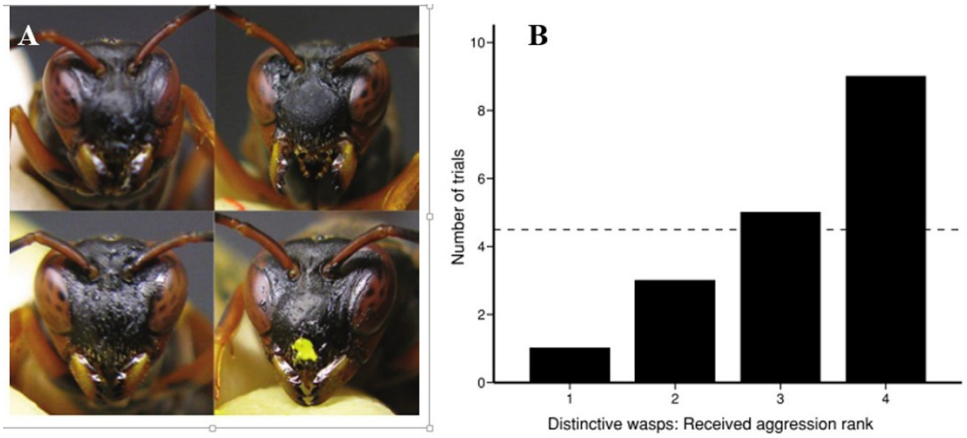

Figure 1.3. Being individually distinctive is beneficial. Faces of *Polistes fuscatus* foundresses were manipulated so three wasps had a common, indistinguishable appearance and one wasp had a unique appearance (Fig. 3A lower-right image). Distinctive wasps received the least aggression (1= most, 4= least) in a disproportionate number of trials, indicating that a unique appearance that allows individual recognition is beneficial. Dotted line represents the expected aggression received by chance. (Fig. 3B). Taken from Sheehan and Tibbetts 2009.

Complexity of individual recognition

There is enormous variation in the cognitive demands of individual recognition. Learning and remembering a few individuals for a short time may be relatively simple compared with learning many individuals, maintaining memories for long periods, or associating multiple types of information with individual identity. In this section, cognitively challenging aspects of individual recognition are described.

Most early individual recognition research examined how animals recognize others using a single cue in a single sensory modality. However, more recent work has shown that many

animals integrate multiple cues from the same or different sensory modalities during individual recognition. For example, golden hamsters (*Mesocricetus auratus*) have multiple odor glands and hamsters generalized between different scents from the same individual. The authors suggest that males formed integrated representations of conspecifics that incorporate multiple odors (Johnston and Bullock, 2001). Other species form cross-modal representations of known individuals by combining unique auditory and visual cues. For example, ring-tailed lemurs (*Lemur catta*) can produce multisensory representations by matching olfactory and visual signals to individually recognize conspecifics, though the specific proximate mechanisms that are used for sensory integration are not yet clear (Kulahci, Drea, Rubenstein, and Ghazanfar, 2014).

Many animals can remember individual conspecifics after a lengthy separation. For example, fur seal pups (*Callorhinus ursinus*) remember their mother's unique calls for at least 4 years (Insley, 2000). Dolphins (*Tursiops truncatus*) may be able to remember unique conspecifics for decades (Bruck, 2013)**.** Male hooded warbler birds (*Wilsonia citrina*) use song to recognize their neighbors individually and are able to retain the memory after being separated for 8 months. During the separation, birds do not sing and migrate to Central America (Godard, 1991). Ravens (*Corvus corax*) remember previous individual songs after up to a three year separation (Boeckle and Bugnyar, 2012).

Long-term individual memory may be particularly important in societies with fission-fusion dynamics, where individuals meet after unpredictably long periods of separation. Memory is also affected by the context in which the recognition is effective. For example, the mantis shrimp *Neogonodactylus bredini* can remember conspecifics for four weeks, perhaps because this amount of time correlates with their reproductive periodicity because female's receptivity and male-female pairing is timed with the full moon (Vetter and Caldwell, 2015).

The cognitive challenges of individual recognition are also influenced by the number of individuals that are learned. African elephants (*Loxodonta africana*), have large recognition networks, as adult females can recognize up to 100 other females (McComb, Moss, Sayialel, and Baker, 2000). Elephant females can recognize their own family, as well as approximately 14 other families they regularly encounter. Such impressive networks of vocal recognition may be typical of long-lived species that have fission-fusion social systems and long-distance vocal communication.

Some species learn to identify surprisingly few individuals despite apparently high levels of social complexity. For example, Gelada baboons, *Theropithecus gelada,* use vocalizations for individual recognition only within the small social group known as a 'unit'. They do not vocally recognize other males, even males from their own band with whom they have extensive social overlap (Bergman, 2010).

It is often difficult to assess whether memory constrains individual recognition. Does reduced recognition in Geladas occur because males have poor memories, reduced motivation, or little social need for individual recognition? In a creative experiment, Stoddard tested whether memory constrained the ability of song sparrows (*Melospiza melodia*) to learn neighbor's songs. Song sparrows can memorize the full song repertoire of new neighbors without a decline in overall learning performance and without forgetting the old songs, even when the number of songs learned approached the number of songs a song sparrow would have to know to make accurate discriminations across neighbors (Stoddard, Beecher, Loesche, and Campbell, 1992).

Another factor that influences the complexity of individual recognition is the types of information associated with individual identity cues. For example, baboons (*Papio hamadryas ursinus*) simultaneously classify other females according to dominance rank and matrilineal

kinship. Associating both rank and kinship information with the unique calls of conspecifics increases the challenge of individual recognition as well as the functional complexity of social interactions (Bergman, Beehner, Cheney, and Seyfarth, 2003). To our knowledge, there is no evidence for this type of two-dimensional classification outside primates, so research in additional taxa will be important.

Some nonhuman primates use individual recognition to keep track of others' relationships. For example, baboons are more likely to approach and tolerate an opponent if they hear a 'reconciliatory' grunt of a close relative of the opponent. However, 'reconciliatory' grunts from individuals unrelated to the opponent have no effect on behavior. This behavior means baboons keep track of relatedness between other individuals rather than merely remembering their own relatives. Recognition of others' relationships will dramatically increase the amount of information individuals need to remember. For example, in a group of 80 individuals, a baboon who keeps track of their own relationship with each group member may only recall two pieces of information about the 79 other group members: are they related or unrelated to the focal individual and are they dominant or subordinate to the focal individual? In that same group of 80 individuals, there are 3160 possible dyad and 82160 triad combinations (Platt et al., 2016). Current data suggests baboons can identify the relatedness and relative rank in all those potential groupings, a truly outstanding feat. In the future, additional work will be important to understand how well non-human species keep track of other's relationships.

Cognitive and sensory adaptations in receivers to facilitate accurate recognition

Learning and remembering many unique individuals is thought to be cognitively challenging. As a result, some species have cognitive adaptations to facilitate accurate individual recognition. These adaptations fall into three categories: 1) general increase in cognitive capacity

in species with individual recognition, 2) specialized increase in cognition related to individual recognition, and 3) sensory adaptations to facilitate assessment of individual identity signals.

First, individual recognition may produce generalized increase in cognitive capacity. The social intelligence hypothesis proposes that the benefits of being able to learn and remember multiple individuals and social relationships has selected for increased cognitive capacity in many social taxa (Dunbar, 1998). Between species comparisons have provided consistent support for the social intelligence hypotheses. Across many taxa, species with more complex social behavior have larger brains or brain regions than species with less complex social behavior (e.g. primates, hyenas, lemurs, ungulates, carnivores, cetaceans, birds). For example, a comparative analysis in nonhuman primate species found that relative neocortical volume is linked with social group size, but not ecology, suggesting that individual social relationships are a key factor that shapes brain size evolution (Dunbar, 1992).

Other analyses have found no relationship between brain size and social complexity. For example, a comparative study on brain size and social complexity in 29 species of Vespids (including solitary and social wasps) showed that brain size does not correlate with colony size, social structure (hierarchies), and type of nest foundation (solitary vs social) (O'Donnell et al., 2015). The authors hypothesized that insects can perform relatively complex tasks with minor adjustments of neural circuitry. Further, many highly social insects have been selected to have more specialized brains rather than larger brains. More generally, there is a growing appreciation that understanding the relationship between social behavior and cognition requires nuanced assessment of social complexity that is relevant to the taxa of interest (Platt et al., 2016).

Second, some species have specific cognitive adaptations that facilitate individual recognition. *Polistes fuscatus* paper wasps use individual face recognition and are specialized

for face learning; wasps learn to identify conspecific faces more quickly and accurately than other visual information. In contrast, paper wasp species without individual recognition perform poorly at face learning. A closely related wasp species that lacks individual face recognition, *P. metricus*, lacks face specialization and has trouble learning conspecific faces. The divergence in face learning between closely related wasp species suggests that face specialization in *P. fuscatus* is an adaptation to facilitate individual recognition (Sheehan and Tibbetts, 2011). Face specialization in paper wasps may be associated with some neural changes in the antennal lobe and the mushroom body sub-compartments. However, there appears to be no major optic lobe specialization in species with visual individual recognition, suggesting that the visual processing capabilities of paper wasps might be preadapted to discriminate complex visual features, such that the ability to discriminate facial markings could require relatively small changes in their neuronal substrate (Gronenberg, Ash, and Tibbetts, 2007).

Neural specialization for individual recognition has been best-studied in primates. Humans are experts at using facial features for individual recognition. Humans can learn and remember many individuals over their lifetime and only require short exposure to form individual memories. Humans also use configural processing to learn faces, as the spatial arrangement of facial features is integrated into a single 'face image' rather than recognition being based on the component parts of a face. Finally, humans have neural specialization for face learning; there is a network of distributed neural regions that respond specifically to faces, not other visual stimuli. Damage in specialized area can produce individuals who are unable to recognize individual faces (Tsao and Livingstone, 2008). Non-human primates also use faces for individual recognition and have some specialization for face learning, including face selective neural responses in certain regions of the brain. Some primates also use configural processing to

discriminate between individual faces, though most primates rely less on configural face processing than do humans (Leopold and Rhodes, 2010).

Finally, some animals have sensory adaptations to optimize assessment of individual identity signals. Paper wasps with visual recognition have larger facets in the eye's acute zone than species without visual recognition. Larger facets improve resolution of small images, such as wasp facial signals. Therefore, the wasps' sensory systems may evolve to optimize signal assessment (Fig. 1.4) (Sheehan, Jinn, and Tibbetts, 2014). In mice, odors (Major Urinary Protein) signal individuality. The neurons in the vomeronasal system (olfactory system) are activated selectively by odors that signal individuality and gender, suggesting an olfactory adaptation for individually recognition of conspecifics (Cheetham et al., 2007).

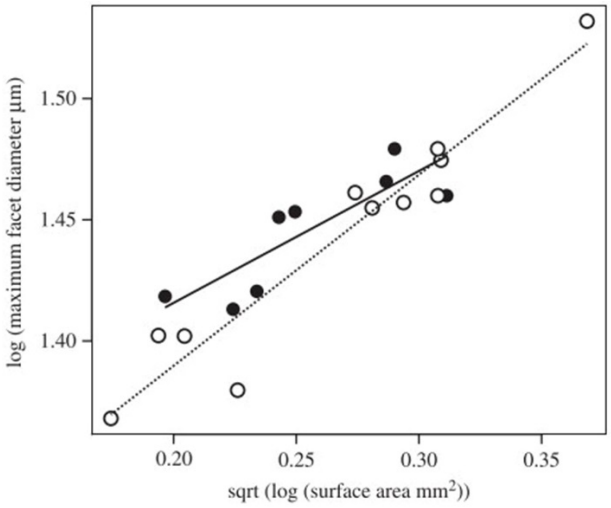

Figure 1.4. *Polistes* wasps species that exhibit visual signal also exhibit larger facets than expected in the eye acute zone. Filled circles: visual signaling species, open circles: non-visual signaling species. Having larger facets is considered an adaptation to facilitate recognition via visual signals. Taken from Sheehan et al. (2014).

Individual recognition from the signaler's perspective (individual identity signals)

Signals vs cues

Individual recognition can occur via signals or cues. Signals and cues are similar in that both modify the behavior of receivers. The key difference between signals and cues is that signals benefit the signaler, while cues do not (Searcy and Nowicki 2005). For example, human faces are signals that convey information about individual identity to receivers. Humans likely benefit by having unique, easily recognizable faces. As a result, human facial variation has been selected to optimize receiver recognition and this selection has shaped human facial variation as well as the genetic regions that code for human facial variation (Fig. 1.5; Sheehan and Nachman 2014). In contrast, human fingerprints are a cue of individual identity. While it is possible to discriminate among individual humans based on fingerprints, variation in fingerprints is a byproduct of development. Fingerprints have not been selected to convey information about individual identity, so fingerprints and the regions that code for fingerprint variation will not have signatures of selection to optimize distinctiveness.

The distinction between signals and cues is important because signals show signatures of adaptive evolution to facilitate receiver responses, while cues do not. The following sections describe the predicted characteristics of individual identity signals.

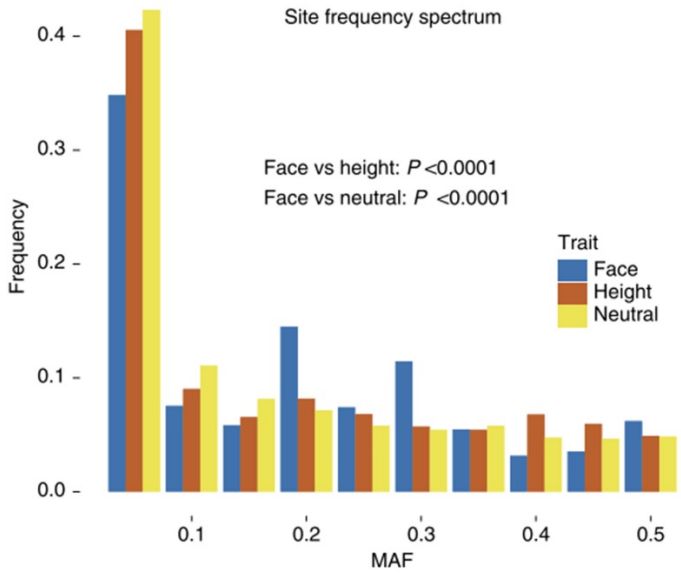

Figure 1.5. Genomic regions associated with facial morphology show evidence of selection for individual identity signaling. Bars represent the proportion of SNPs within each Minor Allele Frequency (MAF). Genomic regions associated with facial morphology have elevated levels of intermediate-frequency alleles when compared with neutral regions or genomic regions associated with variation in height. The higher genetic diversity in face-associated SNPs is consistent with negative frequency dependent selection acting on human facial features. Taken from Sheehan and Nachman (2014).

Negative frequency dependent selection acts on signalers

Individual identity signals are expected to evolve via negative frequency dependent selection (Dale, Lank, and Reeve, 2001). Individuals with rare phenotypes are favored by selection because they are less likely to be confused with other individuals than those with common phenotypes. There is experimental evidence in both *Polistes* paper wasps and guppies, *Poecilia reticulata,* showing that individuals with a rare appearance are favored relative to individuals with a more common appearance. For example, *Polistes fuscatus* paper wasps have highly variable facial patterns that are used for individual recognition (Tibbetts, 2002). Wasps with unique faces that are easy to discriminate receive less aggression than wasps with common, less distinguishable faces. Receiving aggression is costly, so these results indicate that individuals with unique phenotypes benefit by revealing their identity (Sheehan and Tibbetts,

2009; Figure 1.3). Field studies have shown that guppies with rare body coloration benefit by receiving more mates than guppies with a common appearance (Hughes, Houde, Price, and Rodd, 2013). Guppies use body coloration for individual recognition, so the mating benefit may arise through identity signaling if rare guppies are easier to learn and remember than common guppies.

Comparative work provides further evidence that individual recognition selects for increased phenotypic diversity. In paper wasps, mammals, swallows, and bird eggs individual identity signaling is linked with increased phenotypic diversity (Tibbetts, Mullen, and Dale, 2017). This relationship is consistent with the hypothesis that social benefits of being individually recognizable favor increased phenotypic diversity in certain social contexts. For example, computer based model have shown that egg signatures by hosts of the common cuckoo evolve surprisingly fast in the presence of brood parasites. The simulation suggests many hosts evolve unique, highly recognizable egg pattern signatures as a defense against cuckoo egg mimicry (Stoddard, Kilner, and Town, 2014).

Effects of individual identity signaling on phenotypes and genotypes

Because identity signals evolve under negatively frequency dependent selection, identity signals are expected to be highly variable, multimodal, and have low inter-correlation among identity signaling traits. These predictions are developed in detail in the model by Dale and colleagues (Dale et al., 2001). Further, in the taxa that have been tested, there is low intercorrelation among identity signaling traits (Dale, 2000; Tibbetts, 2002). Finally, reliable identity signals are predicted to be stable over time rather than plastic.

Theoretical and empirical work suggest that individual identity signals will be highly heritable and have low condition-dependence. The few individual identity signals studied have high heritability (Dale, 2000). Identity signals are also expected to have low costs associated with signal production and maintenance because phenotypes that are not costly will spread to a higher equilibrium frequency than costly phenotypes. For example, the birds ruff sandpipers (*Philomachus pugnax*) and red-billed queleas (*Quelea quelea*) live in dense aggregations in which individual recognition is key for territory defense. In both species, plumage coloration is highly heritable, and has low production costs (Dale et al., 2001). Similarly, rock hyrax (*Procavia capensis*) use the same highly variable calls for individual recognition across multiple years and these calls are not linked to geographic locations or relatedness (Lee and Eli, 2011). Paper wasps use facial patterns for individual recognition. Facial patterns are fixed during adulthood and facial pattern development is not condition-dependent, as larval diet quality has no effect on facial pattern development (Tibbetts and Curtis, 2007).

Because individual identity signals are highly heritable traits under negatively frequency dependent selection, selection for identity signaling may increase genetic diversity. Positive selection for signaling individual identity may have more widespread genetic effects than other cases of negative-frequency dependent selection because identity signaling favors high levels of variation in multiple, uncorrelated traits (Tibbetts et al., 2017). In contrast, other examples of negative-frequency dependent selection typically maintain a limited number of morphs at stable ratios in the population.

The precise effects of individual identity signaling on the genome will differ based on the genetic architecture of identity signals (Tibbetts et al., 2017). For example, negatively frequency dependent selection acting on individual identity signals will have broader genomic

consequences if signal development is influenced by many, independently segregating loci than if signal development is influenced by epistatic interactions in a small cluster of genes. Population genetic evidence indicates that negative frequency dependent selection acting on loci that encode human facial features influences genomic diversity in humans. Specifically, loci that encode human facial features have higher nucleotide diversity than loci associated with variation in height or neutral, intergenic variation, consistent with human facial features experiencing negative frequency dependent selection (Fig. 1.5; Sheehan and Nachman 2014). Further, the genomic regions surrounding areas that code for face variation also have increased nucleotide diversity, indicating that selection on human identity signals has broader effects on genomic diversity. In contrast, selection for identity signaling in mice has limited genomic consequences. Mice use major urinary proteins (MUPs) as chemical signals of individual identity. Although variation in MUPs is maintained by negative frequency dependent selection, identity signaling in mice has little overall effect on the genome because a small number of clustered loci produce the overall MUP signature (Sheehan et al., 2016).

Complex signals integration: nestmate recognition

Recognizing others allows animals to stablish social hierarchies, find a mate, recognize their own species, and even to differentiate preys and predators (Tibbetts and Dale, 2007). Past work on recognition has focused on signals in isolation, much of this work has asked how a single signal is recognized. However, there is a growing appreciation that animals often have multiple signals across different modalities (e.g. visual and chemical) (Hebets and Papaj, 2005). For example, the domestic cat marks its territory using visual (scratch trees) and chemical signals (urine spray) (Feldman, 1994). Indeed, there is information that cannot be delivered using only one signal, multiple signals appear to be an important mechanism to deliver information between animals

(Higham and Hebets, 2013). Surprisingly, little is known about how multiple signals in social species behave together for recognition, and how the receiver perceives them (Hebets, 2011). Understanding the mechanisms of recognition by multiple signaling is important because it forms the basis of complex social behaviors including cooperation.

Three hypotheses have been proposed for why animals have multiple signals. First, multiple signals could convey different information between them (content-based hypothesis). For example, one signal could indicate species identity information while the other provides information about signaler quality (Hebets and Papaj, 2005). Second, signals can also interact between each other (inter-signal interaction hypothesis). For example, interaction of multiple signals can amplify the information that each signal conveys, or the combination of signals can produce emergent properties that are better perceived by the receiver. And third, multiple signaling could be associated to the efficacy with signals travel through the environment and are received (efficacy-based hypothesis). For example, signals can be redundant because if one is lost in the environment the other could serve as a "back up" (Hebets and Papaj, 2005). However, empirical evidence on these hypotheses is not abundant mostly in complex social contexts such as in eusocial species (Higham and Hebets, 2013).

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CHAPTER TWO

INTRASPECIFIC GEOGRAPHIC VARIATION IN INDIVIDUAL RECOGNITION: PHENOTYPIC VARIATION IN SIGNALERS AND RECEIVERS

ABSTRACT

The geographic distribution of a species influences the communication systems used by the species. However, the study of the geographic co-variation of the phenotypes that make possible communication present in signalers and receivers of the same species has not received much attention. Here, we analyze the intraspecific geographic variation in individual recognition in signalers and receivers exhibited by the paper wasp *Polistes fuscatus*. Individual recognition in *P. fuscatus* is mediated by signals of individual identity in the signaler (e.g. facial and abdominal color marks) and recognition abilities in the receiver to remember and behave in a unique way toward the signaler (e.g. memory). Thus, for a population to be considered as exhibiting individual recognition, the population should show high within-population facial and abdominal color mark variation in the signalers and individual recognition abilities in the receivers. We sampled six populations of *P. fuscatus* wasps across 650 km from Pennsylvania to Michigan, USA, and studied the natural within-population diversity of signalers' facial and abdominal color marks, and receivers' abilities to individually recognize conspecifics. We found that the diversity of signalers' facial and abdominal color marks significantly vary across populations, where two populations exhibited higher within-populatio facial and abdominal color marks diversity than the other

populations. From the receivers' perspective, we found that only two out of the six studied populations were able to individually recognize conspecifics. These results suggest that there is intraspecific geographic variation in individual recognition in *P. fuscatus*. Therefore, social behaviors that strongly influence the social structure of the colony can be flexible and can vary across populations. Complex social behaviors in eusocial species could be more flexible than previously expected.

INTRODUCTION

Geographic intraspecific variation in animal communication is a remarkable phenomenon present from mammals to fishes to insects (Foster and Endler 1999). However, it is unclear how phenotypes in both signalers and receivers that are associated with the communication system respond to such a variation (Foster, 2013; Wilcznski & Ryan, 1999). One of the most interesting examples of geographic variation in communication is song dialects in oscine birds. Geographically distant populations of these birds show differences in signal production such as the preponderance of syllables in each song, as well as receivers' population-level preferences for certain song dialects (Baker & Cunningham, 1985; Toews, 2017). Theoretically, intraspecific communication posts a challenge to behavioral biology because there are two entities interacting (signaler and receiver) where their morphological, physiological, and behavioral components are often separable (Wilcznski & Ryan, 1999). Each of these components can be under different selective pressures and mechanistic processes. One can think that given this tight relationship between signaler and receiver, their phenotypes that allow communication must be invariant or species-typical (Foster, 1999). However, empirical evidence on geographic variation in intraspecific communication has been reported in vertebrate and invertebrate taxa. For example, cricket frogs (*Acrins prepitans)* show geographic variation in the dominant frequency of male

calls (Keddy-Hector, Wilezynski, & Ryan, 1992; McClelland, 1996). Interestingly, female auditory systems change geographically in the same general way as the male call frequencies do, as well as female preference for male calls between populations. (Ryan, 1990). Yet, we do not know how the correlated evolution in phenotypes between signalers and receivers is affected when there is intraspecific geographic variation in communication.

In intraspecific communication systems, signaler's and receiver's phenotypes are expected to coevolve (Wilcznski & Ryan, 1999). An efficient communication interaction depends on the production of a signal by the sender and the receiver's abilities to produce a response based on the signal (Tibbetts & Dale, 2007). Thus, a communication interaction with a signal production would not be successful without a receiver's response or vice versa. For example, in mate choice, assessing the signals of the sender and producing an appropriate response is crucial for reproduction (Hebets & Papaj, 2005). Therefore, this causal interaction between signalers and receivers can have fitness consequences and evolutionary implications (Dale, Lank, & Reeve, 2001). This coevolution between sender and receiver makes the study of intraspecific geographic variation in communication particularly interesting because we can then dissect, in specific measurable traits that are present in signalers and receivers, how complex behaviors evolve.

However, there are species-typical signals and sensory systems that do not vary intraspecifically. Most of the communication interactions that are constant intraspecifically are associated with species recognition (Wilcznski & Ryan, 1999). For example, the frequency of frog calls and the level of tuning in their acoustic sensory systems lie in a certain range that is set for the entire species; otherwise, it would be difficult to distinguish etween conspecifics and heterospecifics (Nevo & Capranica, 1985; Ryan & Keddy-Hector, 1992). The same principle applies in birds, for example (Petruskova, O, & Petusek, 2010), mammals (Wich, Schel, &

Vries, 2008), fishes (Endler & Houde, 1995), and social insects (Dapporto, Palagi, & Turillazzi, 2004). This may be due to local variation in species recognition that could lead to reproductive isolation and speciation (Wilcznski & Ryan, 1999).

Individual recognition is a type of communication system that exhibits characteristics that could potentially be used for the study of intraspecific variation in complex social behaviors. Individual recognition occurs when receivers discriminate a signaler from others based on the signaler's unique characteristics and associate the unique characteristics with individual-specific information about the signaler (Tibbetts & Dale, 2007). This form of recognition is used mainly for discrimination of siblings, mates, friends, foes, or neighbors (Cely & Tibbetts, 2018; Tibbetts & Dale, 2007). Receivers and signalers in this recognition system have particular adaptations that allow them to recognize and interact with each other (Tibbetts & Sheehan, 2013). For instance, signalers must have individual identity signals such as high levels of color pattern variation (just like human faces are all different so we can recognize others) (Dale et al., 2001; Tibbetts, Mullen, & Dale, 2017). Receivers are equipped with sensory and recognition adaptations such as learning abilities (e.g. memory) to perceive and remember the signaler's signals (Tsao & Livingstone, 2008). These characteristics make individual recognition a complex type of communication, with high population-level phenotypic diversity in the signalers, and elaborate sensory systems and recognition abilities in the receivers (Tibbetts & Dale, 2007). In contrast to the relatively abundant literature on how individual recognition is distributed and expressed across animal species, few studies have explored how individual recognition is distributed within populations of a single species. We know that individual recognition can occur from solitary to eusocial animals. We know in what social contexts individual recognition is more likely to occur (e.g. territoriality, dominance interactions, cooperation, etc.). We also know that some animals

can even individually recognize individuals from other species, such as mockingbirds with humans, but still we lack evidence on geographic intraspecific variation of individual recognition (Cely & Tibbetts, 2018).

Geographic variation in individual recognition in other animals has shown variation in signalers' phenotypes, but receivers' responses have not been extensively explored under that context. For example, long calls of orangutans from several populations in Sumatra and Borneo have shown that there are variations in signalers' callings (pulse per call, call speed, call duration, bandwidth, pulse duration, and dominant frequency) across the populations but the receivers' responses to confirm individual recognition have not been undertaken (Delgado, 2007). The study of the receivers' behavioral responses for individual recognition is not common in literature, and they are almost nonexistent when studying geographic variation of individual recognition (Cely & Tibbetts, 2018; Tibbetts & Dale, 2007; Tibbetts, Sheehan, & Dale, 2008). Despite the importance of these type of studies, we lack the information necessary to analyze the coevolutionary patterns of signalers and receivers as a whole in geographic distant populations.

The paper wasp *Polistes fuscatus* (Fabricius) is an ideal model for the study of geographic variation in individual recognition. *P. fuscatus* is a eusocial species that exhibits individual recognition; each wasp has individual-specific signals (e.g. face and abdominal color marks) that allow other wasps to individually recognize and treat her in a unique way (Tibbetts, 2002). Receivers have acute vision and learning abilities (can remember previous encounters for up to one week) (Sheehan, Jinn, & Tibbetts, 2014; Sheehan & Tibbetts, 2011). In addition, the geographical distribution of *P. fuscatus* ranges from southern Canada to the United States to Central America (Miller et al., 2018; Santos, Payne, Pickett, & Carpenter, 2014; West-Eberhard, 1969). Studies on individual recognition in natural populations of *P. fuscatus* have been

performed predominantly in populations of southeast Michigan and upstate New York, USA (Injaian & Tibbetts, 2013; Tibbetts, 2002). Thus, we saw the potential for geographic variation in this animal model that has not been previously investigated.

Here, we evaluate the geographic variation in the behavior of individual recognition in populations of *P. fuscatus*. We hypothesize that individual recognition will have variations across geographically distant populations, which have an impact in the natural phenotypic diversity (in signalers and receivers) of *P. fuscatus*. We predict that traits that allow for individual recognition to occur, such as diversity in signalers' visual features (faces and abdomen marks) and receivers' recognition abilities, will vary across the studied populations. We provide empirical evidence demonstrating that there is indeed intraspecific geographic variation in individual recognition in *P. fuscatus*, and that phenotypic diversity (morphological and behavioral) is linked with this variation in sender phenotypes and receiver responses.

METHODS

(a) Specimens collection:

Foundresses ("queens") of *P. fuscatus* wasps were collected alive in the last week of May in 2016, 2017, and 2018 following a transect composed by six populations from Roth Rock State Forest (Pennsylvania) to the University of Michigan's Edwin S. George Reserve (Michigan) in the USA (Figure 2.1 and Table 2.1 for full details on locations). Wasps were brought alive to the University of Michigan for the experimental essays. They were fed *ad libitum* with sugar and water. Foundresses were housed in separate containers with their respective nest. Each wasp was painted on the dorsal side of the thorax with Testors enamel paint for further observer

identification. Lights and temperature were controlled to replicate normal environmental conditions.

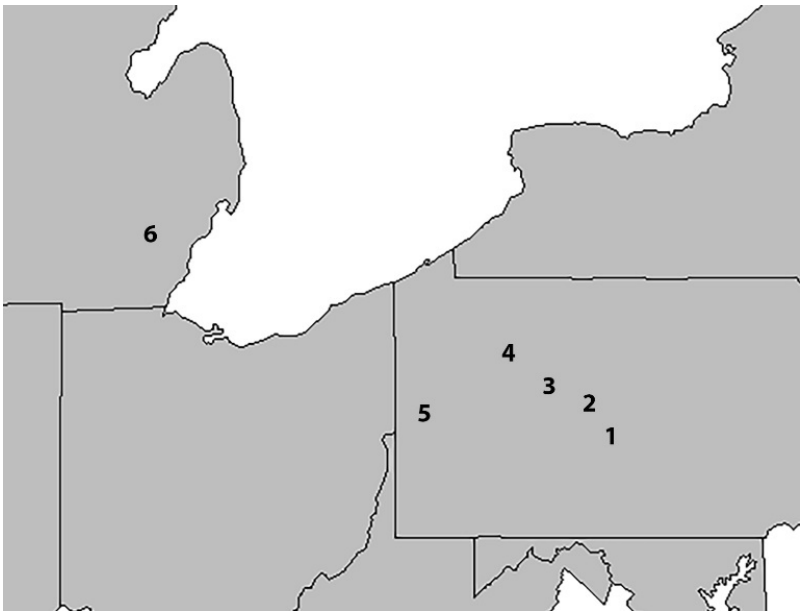

Figure 2.1. Distribution of sampling in the present study, from State College, Pennsylvania (1) to Pinckney, Michigan (6), USA. Each number represents a population. For the geographic coordinates refer to table 1.

Table 2.1. Locations and codes for each of the population sampled with their respective number of individuals used in each analysis in this study.

Population	Location	Geographic coordinates	Elevation (meters)	Number of individuals used for visual variation	Number of individuals used for IR
1	Roth Rock State Forest	40.637052, -78.074672	588	124	19
2	Black Moshanoon State Park	40.913807, -78.067813	585	77	12
3	S. B. Elliot State Park	41.113026, -78.525817	646	48	19
4	Clear creek State Park	41.327238, -79.092582	397	76	22
5	Moraine State Park	40.939271, -80.097115	382	Low sample size	21
6	Edwing S. George Reserve	42.458422, -84.011562	295	52	42

(b) Determination of the signalers' color mark diversity (face and abdomen):

The face and abdomen of each collected wasp were photographed. With the pictures taken per wasp, we described the variation in color marks that were present in each population. We used character states to score the color mark variation present in the face and abdomen. These character states that described the distribution of the colors present in the face and abdomen (from 0 to 4, see table 2.2) were the extent of the frons yellow stripe, the extent of the yellow stripe along the clypeus, and the extent of the black and brown colors in the clypeus (Table 2.2 and figure 2.2). For the abdomen, we scored the number of dorsal tergites that presented yellow stripes. Using those qualitative characters, we generated a phenotype per individual. Thus, each individual had a phenotype description summarizing the character states of the face and abdominal marks (Table 2.3).

Table 2.2. Characters and character states for the facial color marks. Note that for the abdominal marks we only scored the number of dorsal tergites with yellow stripes.

Score	Yellow Frons	Yellow Clypeus	Black Clypeus
0	Absent	Absent	No black observed in the center of the clypeus, a very thin edge of black may be observed
1	Small, unconnected patches of yellow	Small dot of yellow at the tip of clypeus	Small to medium sized dot of black in the center of the clypeus
2	Two yellow patches are connected, but are thin	Thin line of yellow at the bottom of the clypeus, does not extend all the way to the eye	A band of black stretching across the clypeus, occupying less than 75% of the total area
3	Thick yellow marks connected at the midline	Yellow band along the two sides and bottom of the clypeus, resembling a smile	More than 75% of the clypeus area not covered by yellow is covered by black
4	As with 3, but at least as twice as thick	As with 3, but at least as twice as thick	No brown is present, all area of the clypeus that are not yellow are black

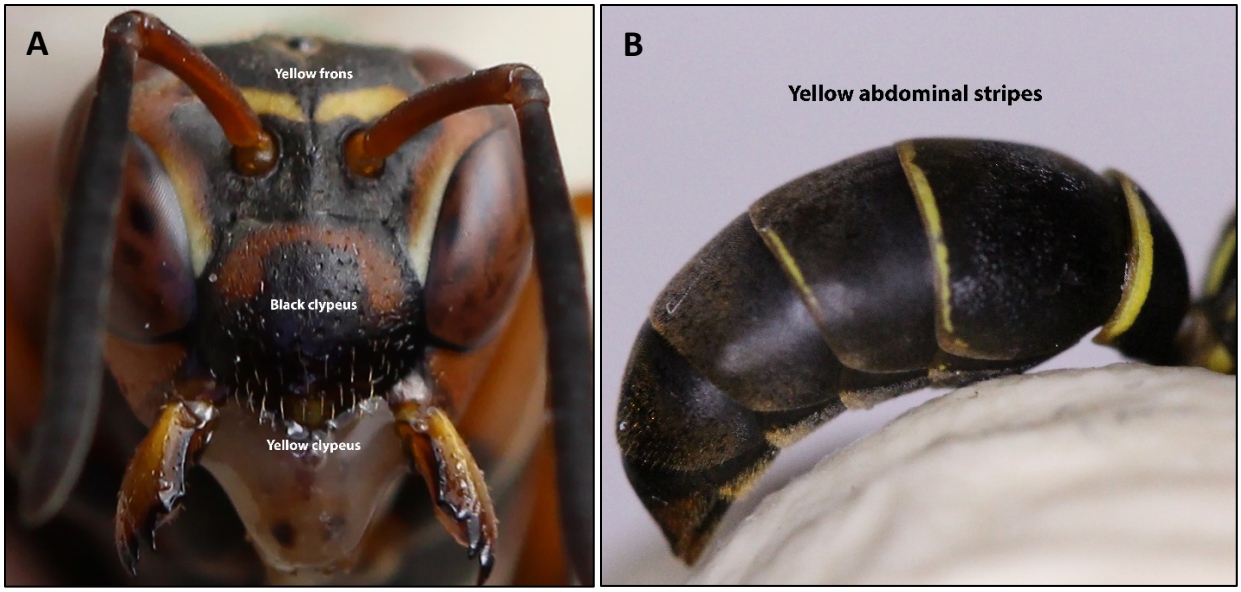

Figure 2.2. Location of the characters used in *P. fuscatus* for the color mark variation analysis. **A.** Frontal view of the face. **B.** Lateral view of the abdomen.

Table 2.3. Example with three specimens showing the matrix obtained from the character states scored to generate the phenotype per individual.

wasp code	# of abdominal segments with yellow stripes	Yellow frons	Yellow clypeus	Black clypeus	Phenotype
C2F30	1	1	2	4	1,1,2,4
CF111	2	0	2	2	2,0,2,2
CF204	3	1	1	4	3,1,1,4

(c) Behavioral experiments for determining individual recognition:

The recognition abilities exhibited by each studied population of *P. fuscatus* were evaluated by experimentally pairing wasps that either had previously encountered each other or not. Then, we determined the levels of aggression and non-aggression displayed by a focal wasp to familiar and unfamiliar wasps (Injaian & Tibbetts, 2013; Sheehan & Tibbetts, 2008). The trials were

performed in transparent plastic containers of 8x8 cm. This container size allows wasps to move freely but also to maintain interactions between them.

The experiment lasted four days. We video-recorded the interaction between the wasps each day. On day one, we placed two foundresses in an empty container and filmed their interactions for 30 minutes. These two initial wasps had no previous encounters. After 30 minutes, we placed these wasps together in a container with water and sugar until the next day; after that, they were returned to their original containers with sugar and water. These two wasps were paired again on day three. Their interactions were video-recorded for 30 minutes as well. If wasps can remember their encounter in day 1, then the aggression level should be lower in day three given their stereotyped dominance agonistic behavior. In day two and four, we paired those initial wasps with other wasps with which they had no previous interactions. This was done to confirm that, if the aggression displayed on day three was lower than on day one, it was not because the wasps were unmotivated or tired. If change in aggression during the experiment is due to factors other than individual recognition, we predict that the aggression showed in day three will not be the lowest aggression level exhibited between all the experiment days.

Behavioral observations: To standardize the levels of aggression per video tape which allow us to compare the aggression exhibited in the trials, we calculated the aggression index. To calculate the aggression index, we ranked the observed behaviors as follows: (0) non-aggressive behavior (pair within a body length distance but no darting, biting or grappling). (1) dart (rapid body movement toward partner). (2) dart with open mandibles. (3) bite. (4) grapple or mounting (most aggressive behavior; the receiver normally accepts submissive positioning). Then, we summed the ranks per video and the result was divided by the total number of interactions (Injaian & Tibbetts, 2013). For example, if the behaviors scored in a given video tape were one

dart, one dart with open mandibles, one bite, and one mounting, then the aggression index would be 7/4 = 1.75. This methodology for behavioral assessment has been used in previous studies on *P. fuscatus* (Dreier, van Zweden, & D'Ettorre, 2007; Tibbetts, Injaian, Sheehan, & Desjardins, 2018).

(d) Data analysis:

Phenotypic diversity: To standardize samples on the basis of sample size or sample completeness and facilitate the comparison of the phenotypic diversity data between populations, we used the Hill numbers or the effective number of species (in this case, phenotypes). Other diversity indices (e.g. Gini-Simpson index) are non-linear, so statistical comparisons between indices can be misleading. Hill numbers transform these diversity indices into effective number of species (Chao, Gotelli, Hsieh, Sander, & Colwell, 2014). For example, if a population has an effective number of species of three and another has nine, we can say that the second population is three times more diverse than population one. This type of conclusion cannot be drawn from the other diversity indices given their non-linearity (Jost, 2007).

To compare the diversity of the signalers' color marks between populations, we produced rarefaction curves and extrapolation of the Hill numbers with order q=2 (the inverse of Simpson's concentration index for diversity). This analysis tells us how many phenotypes per population concentrate most of the abundance or the number of individuals per phenotype. We expect that under this scenario, a population considered diverse will exhibit high richness of phenotypes but at the same time the number of individuals per phenotypes should not be dominated by only one or few phenotypes (Chao et al., 2014). Thus, we chose Hill numbers with order q=2 approach because order q=2 report dominant phenotypes rather than just the diversity of all phenotypes (order q=1) or the typical phenotypes (order q=1). Then we can obtain

confidence intervals of 95% (by bootstrap of 40 replications) to determine how different the diversity is of the phenotypes per population based on these effective number of phenotypes. Note that we used the extrapolated values (predicted), which corrects for sampling effort and completeness. We used the Software R version 3.4.3 package iNEXT for this analysis.

Behavioral assays: We used Friedman's ANOVA to establish if there were differences in aggression levels between the days of the experiment, per population. The dependent variable was aggression index and the independent variable was the day of the experiment. When the overall model was significant, Post-hoc least significant difference (LSD test) was performed for the pairwise comparisons of the aggression levels between days, per population. Significance at the level of $p < 0.05$. We used IBM SPSS version 25. For samples sizes see table 2.1.

RESULTS

(a) Diversity of signalers' color marks (face and abdomen):

There are significant differences in the diversity of facial and abdominal marks present between some of the studied populations. Population two and population six show higher levels of diversity in their face and abdominal color marks than populations one, three, and four (table 2.4). The upper confidence interval for the phenotypic diversity of population three overlaps the lower confidence limit to be considered as having high diversity. However, its confidence interval is very wide (2.159 to 20.791) so this population was considered as a low diversity population. Nevertheless, its wide confidence interval suggests that population three requires more sampling effort to determine an accurate estimation of diversity. The Hill numbers for order q=2 showed that the distribution of the abundance of the phenotypes using the 422 individuals in all populations were concentrated in 9, 11 and 11 phenotypes in population one,

three and four, respectively. In contrast, in populations two and six, the phenotypic abundance was concentrated in 40 and 23 phenotypes respectively (table 1 for the sample size per population) (Fig. 2.3 and table 2.4 for the Hill numbers order q=2 extrapolations and their CI).

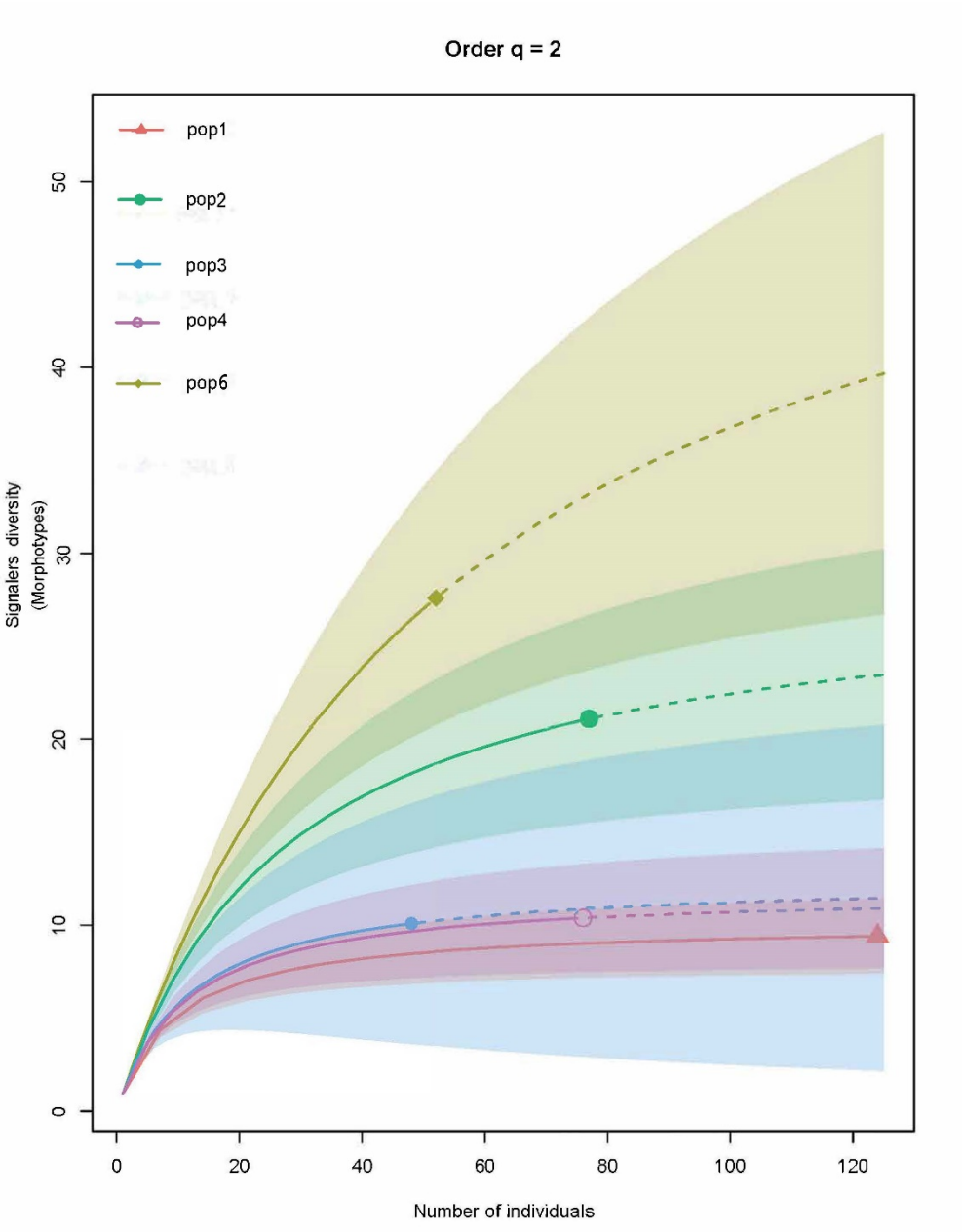

Figure 2.3. Plot of sample coverage for rarefied samples (solid lines) and extrapolation (dashed lines) based on the Hill numbers order q=2. The 95% confidence intervals (shaded regions) were obtained by a bootstrap method based on 40 replications. Reference samples are denoted by the geometric figures (see legend within the figure). Sample size of 422 individuals.

Table 2.4. Diversity estimates with observed and extrapolated samples, per population, based on Hill numbers, order q=2.

	Diversity estimate		Confidence intervals for extrapolated	
Population	*Observed*	*Extrapolated*	*Lower*	*Upper*
1	9.399	9.404	7.17	11.637
2	21.1	23.485	17.575	29.395
3	10.105	11.475	2.159	20.791
4	10.388	10.925	8.101	13.749
6	27.952	39.672	27.584	51.761

(b) Receivers' capability for individual recognition:

If individual recognition occurs, then we expect that the aggression index in day three will be the lowest compared to all the other three days in the experiment. The individual recognition ability was present in two out of the six populations studied (Table 2.5 and fig. 2.4). Individuals from population five and population six exhibited lower aggression toward wasps with whom they had a previous encounter in comparison to wasps that they just met (Fig. 2.4). For these two populations, the aggression index exhibited in day three was significantly lowest in the four days of the experiment (Table 2.5). In contrast, populations one, two, three, and four did not show a difference between known and unknown wasps (Table 2.5 and fig. 2.4). For these four populations, day three was not statistically the highest or the lowest in terms of aggression index in the four days of the experiment (Table 2.5). Note that even though the Friedman's ANOVA shows that there are almost significantly differences between the days for population three, day three was not the different one, which does not support the prediction for individual recognition (Fig. 2.4). Instead, day two was the one with the lowest aggression value, which is not consistent with the pattern expected if the population exhibited individual recognition. In addition, the aggression index between day one and day four of the experiment was the same for all the

populations. This indicates that wasps' aggression across days was not affected by lack of motivation or tiredness (Fig. 2.4).

In summary, there is intraspecific geographic variation in individual recognition, from the signalers' and the receivers' perspectives, across the studied populations in *P. fuscatus* (Table 2.6).

Table 2.5. Friedman's ANOVA for each of the population in the individual recognition experiment. Significance at the level of $p < 0.05$.

	Friedman's ANOVA			
Population	**Chi-Square**	**df**	**Sample size**	**p value**
1	5.463	3	19	0.141
2	3.655	3	12	0.301
3	7.221	3	19	0.061
4	2.683	3	22	0.443
5	9.290	3	21	0.026
6	18.240	3	42	<0.001

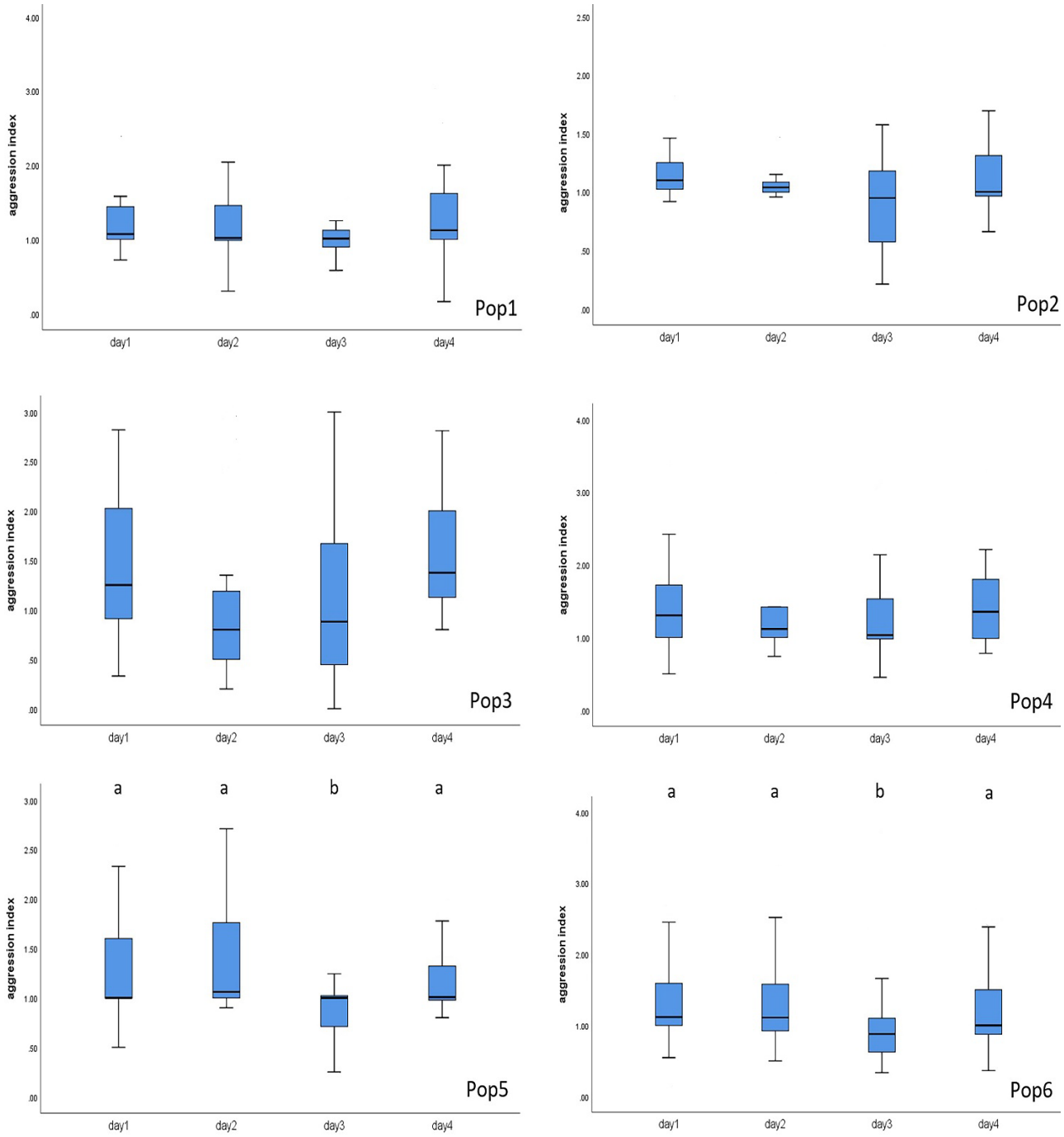

Figure 2.4. Boxplot of the means of aggression index in each of the populations sampled. Only population five and six show differences in aggression index across days of the experiment (significant after Friedman's ANOVA); we then performed pairwise comparisons of the aggression index between days per population using Post-hoc least significant difference (LSD test) on populations five and six. Different letters denote significant differences. Significance at the level of $p < 0.05$. Data from population six is adapted from Sheehan and Tibbetts (2008). Box: first quartile, median and third quartile. Whiskers: minimum and maximum values.

Table 2.6. Summary of the results for both experiments per population.

POPULATION	COLOR MARK VARIATION (signalers)	INDIVIDUAL RECOGNITION ABILITY (Receivers)
1	low	not
2	high	not
3	low	not
4	low	not
5	poor data	yes
6	high	yes

DISCUSSION

Our results provide empirical evidence suggesting that there is intraspecific geographic variation in individual recognition in *P. fuscatus*. The diversity analysis of phenotypes showed that signalers have differences across populations on the diversity level of the facial and abdominal color marks. Having high within-population color marks in signalers is important for individual recognition because individual recognition favors rare phenotypes via negative frequency-dependent selection. Populations one, three, and four have significantly lower facial and abdominal color marks diversity than populations two and six. From the receiver's perspective, there is also geographic variation in the ability to individually recognize conspecifics. There are populations where wasps treat familiar conspecifics in a unique way, such as populations five and six, while the other studied populations cannot.

Taken together, these results suggest that complex forms of communication, such as individual recognition, that depend on the coevolution between signalers' phenotypes and receivers' cognition are not species-typical; instead, they show flexibility. Because of the cognitive complexity and the potential costs of individual recognition, the intraspecific geographic variation in individual recognition may not sound plausible (Injaian & Tibbetts, 2013). This finding is interesting because we are showing that traits associated with complex

behaviors do not evolve as a unit; rather, they coevolve in different ways and populations, creating mosaics of different behaviors and phenotypes.

Behavioral traits are considered to exhibit remarkable plasticity; therefore, the geographic variation in such traits can be immediately responsive, which can influence evolutionary processes (Foster, 2013; Krützen, Willems, & Van Schaik, 2011). For example, the courtship behavior in threespine sticklebacks (*Gasterosteus aculeatus*) rapidly changes depending on the limnetic or benthic ecotype (Foster, Shaw, & Robert, 2008). Similarly, feeding behavior in grasshoppers (*Melanoplus*) changes depending on the differences in hardness or nutrient availability in plantations they find (Thompson, 1992). Thus, geographical variation in complex behaviors that depend heavily on learning abilities such as individual recognition may not be rare.

Performing individual recognition requires learning large amounts of information, retrieving it, and being able to behave accordingly to produce a unique response toward the particular signaler (Cely & Tibbetts, 2018). However, studies on brain composition in *P. fuscatus* have shown that the ability to discriminate facial markings could require relatively small changes in their neuronal substrate (Gronenberg, Ash, & Tibbetts, 2007). In addition, preliminary data have shown that synaptic complexes in the mushroom bodies (major site of sensory integration in the insect brain) are not different between *P. fuscatus* wasps with and without experience facilitating individual recognition (in prep). Moreover, recent studies have shown that *Polistes metricus*, a species without the capability of individual recognition (Sheehan & Tibbetts, 2010), improve their ability to learn visual signals of identity if they have been reared with *P. fuscatus* wasps (Tibbetts, Pandit, & Nondorf, 2018). Furthermore, *P. fuscatus* exhibit intraspecific variation in individual recognition across castes, where foundresses are better at individually

recognizing conspecifics than workers, further supporting plasticity in these wasps (Injaian & Tibbetts, 2013). Here, we showed the flexibility of individual recognition in *P. fuscatus* in a range of 650 km. Thus, individual recognition might be a behavioral task that does not require large brain specializations, and that experience and geographic distribution may play an important role in facilitating flexibility in this behavioral phenotype. For example, population one exhibits about four times less variation in color marks than population six does, and at the same time, receivers in population one cannot individually recognize conspecifics while wasps from population six can perform such a task.

Different pressures in terms of social requirements could explain why some populations exhibit individual recognition and why other populations do not. *P. fuscatus* rely on a strict hierarchical society for the maintenance of their social structure, which is mediated by agonistic interactions (West-Eberhard, 1969). It has been hypothesized that individual recognition plays an important role in this task (Dale et al., 2001). Dale's et al model states that given the high costs of aggressive interactions, individuals with rare and recognizable phenotypes will benefit during social interactions by receiving fewer aggressions. In fact, past research has shown that *P. fuscatus* individuals that display individual recognition exhibit fitness benefits for having unique phenotypes (e.g. being recognized and treated in a unique way by nestmates) (Sheehan & Tibbetts, 2009). In addition, individuals that are injured after fights with nestmates exhibit injuries that reduce their performance in nest activities such as foraging or brood care (Jandt, Tibbetts, & Toth, 2014; West-Eberhard, 1969). Consequently, it could be surprising that individual recognition, which brings individual and group benefits, is present in some populations of *P. fuscatus* but not in others.

The social configuration of the populations lacking individual recognition in *P. fuscatus* might be different than the populations that have individual recognition, resulting in different pressures toward the interaction between signalers and receivers. In orangutans, for example, behaviors that are inherent in social organization such as sociability, susceptibility to social stress, cultural repertoire, male development arrest, or duration of consortship exhibit geographic variation (Schaik, Marshall, & Wich, 2010). However, we have not studied the social organization in the colonies of populations that do not exhibit individual recognition in *P. fuscatus*. This is an area that deserves further exploration to answer the proximate causes of this geographic variation. However, from the present behavioral and morphological analysis, it appears that geographic distances (e.g. isolation by distance) could be a strong force for the development of variation in individual recognition in *P. fuscatus*. Note that in the next chapter, we will analyze the fitness and genetic consequences of geographic variation in individual recognition in *P. fuscatus*.

Testing individual recognition in social contexts is challenging because researchers must determine not only the individual identity of the signalers but also the signalers' unique responses toward the signaler (Tibbetts & Dale, 2007). When the receiver response is not tested, it is unclear if individual identity signals are used for individual recognition. In several taxa, there is evidence of geographic variation in signals that are thought to be associated to individual recognition, but the receiver response has not been assessed. For example, in Tree pipits (*Anthus trivialis*), the repertoire composition at the level of syllables varies across populations, and its in-population variation is so high that it is thought to be for individual recognition (Petruskova et al., 2010). This same type of evidence where there is geographic variation in signalers but the receivers' response has not been tested to confirm individual recognition can be seen in for

example the leaf-nosed bat (*Hipposideros larvatus*) and orangutans (*Pongo* spp), which makes the need for more empirical evidence on receivers an area of attention (Delgado, 2007; Jiang, You, Liu, & Feng, 2009).

Overall, the results of this study indicate that signalers' phenotypes and receivers' behaviors are flexible and can change across geographically distant populations. We found that the diversity of facial and abdominal marks in the signalers are not constant for all populations; rather, this phenotypic diversity is highly variable across the studied populations. In addition, receivers' behaviors (individual recognition) show similar patterns as signalers in terms of variation. This behavior is present in some population but absent in others. This variation in signalers and receivers shows that complex behaviors such as individual recognition can be flexible even across distances of hundreds of kilometers. Our results suggest that geographic variation could be an underappreciated factor facilitating the evolution and diversity of complex behaviors in animals. Studying both the perspective of receivers and signalers when assessing individual recognition, as was done in this study, may provide insights on the natural variation of phenotypes and behaviors. However, research using both perspectives is needed in different taxa to test how flexibility in behaviors affects the evolution of phenotypes and vice versa.

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CHAPTER THREE

INTRASPECIFIC GEOGRAPHIC VARIATION IN INDIVIDUAL RECOGNITION: GENETIC ASSESSMENT

ABSTRACT

Understanding the genetic basis of social behaviors is the focus of a growing literature with a variety of novel genetic approaches. The study of the genetic basis of phenotypes such as behaviors is important in modern evolutionary ecology because recently developed tools to analyze genetic data allow us to explore punctual genetic changes, giving us key insights on how microevolution works. Despite this growing interest in the field, few studies have investigated the genetic basis of the geographical variation of complex social behaviors such as individual recognition. Here, we explore the genetic basis of geographic variation in individual recognition in six populations of the eusocial wasp *Polistes fuscatus*, from Pennsylvania to Michigan, USA, by using a genome reduced-representation approach (ddRAD-seq). First, we evaluated the genetic diversity and genetic structure of the six populations. Fst values suggest that there is gene flow between the populations, and the Mantel test was not positive for isolation by distance. In addition, measurements of genetic diversity between populations were equivalent. Second, we performed a GWAS to find candidate loci under selection given the variation of individual recognition across the populations. BayeScan approach did not find signatures of natural

selection on variable loci, suggesting that either ddRAD-seq and BayeScan approach failed in finding loci under selection, or that behavioral flexibility in *P. fuscatus* is maintained via strong gene flow and interconnectivity between the populations, swamping loci under local adaptations. Taken together, these results show that ecological factors such as dispersal and intrinsic factors such as the genetics and ontogenetics in *P. fuscatus* are important factors that may add to the natural variation in individual recognition exhibited in the populations. Finally, we conclude that genome scans such as ddRAD-seq might not be the most appropriate methodology to study loci under selection in local behavioral adaptations.

INTRODUCTION

Understanding the patterns and processes that contribute to the phenotypic diversity in nature is a central topic in evolutionary biology (Foster, 2013). Although descriptions about variations in phenotypes such as behaviors within a single species are available (Foster and Endler, 1999), little is known about the mechanisms associated with the evolution of behaviors between groups of the same species (Gabor, Aspbury, & Rodríguez, 2013). However, with the advent of next-generation sequencing, we are now closer to identifying the genetic basis that underlies phenotypic differences within the same species (Visscher et al., 2017). For example, twin and adoption studies in humans have demonstrated the correlation between certain genotypes and the risk for psychiatric disorders (Budde et al., 2017). Despite wide acceptance that behaviors have a genetic basis, the study of intraspecific variation of behaviors and their correlation with potential genotypes that favor some behaviors over others has not received much attention (Bendesky & Bargmann, 2011). Associations between genomes and behaviors could give us answers about the why and how of intraspecifically variable behaviors.

Complex behaviors such as individual recognition stand as an ideal model for investigating questions regarding the genetic basis of the intraspecific variation in social behaviors. In individual recognition, receivers discriminate a signaler from others based on the signaler's unique characteristics and associate these characteristics with individual-specific information about the signaler (Cely & Tibbetts, 2018). In chapter one, we demonstrated that in *P. fuscatus* foundresses, individual recognition abilities (in signalers and receivers) are variable across a geographic transect. Thus, individual recognition exhibits geographic variation in the phenotypes that allow for this behavior to occur, which at the same time could represent genetic differences underlying the expression of individual recognition.

Few studies have investigated the genetic basis of intraspecific geographic variation in complex social behaviors in animals. The environment present in geographically distant populations of the same species can vary; thus, behavioral and genetic divergences are expected (Foster, 2013). For example, in the Rainbow Trout (*Oncorhynchus mykiss*), populations that are in low-risk environments are less active than populations that are in risky environments that require more exploration. In these fishes, these dissimilar behaviors are linked with genes associated with risk-taking behaviors (Thomson, Watts, Pottinger, & Sneddon, 2012). Understanding the genetic basis of intraspecific variation in behavior is important for determining how social behaviors evolve (Wilcznski & Ryan, 1999).

Population contrasts within a single species is especially valuable for inferring the genetic basis of behavioral differences. This is because the divergence time within the same species is expected to be shorter than the time of divergence between species. This is attractive for studying the evolution of specific traits because the diverging populations might still be living in a similar environment; furthermore, there are fewer traits that differ between populations than between

species (Foster, 1999). Comparing populations of the same species can also give us insights on early stages of speciation, and the role that genes and environment play in certain phenotypes (Selander, 1970). Hence, comparing geographically distant populations of the same species is a powerful tool for determining the evolutionary patterns of behaviors. Here, we build on the previous finding that individual recognition in *P. fuscatus* is variable across geographically distant populations by studying the genetic differences that explain the variation in individual recognition across populations.

Genome reduced-representation approaches, such as double-digest restriction-associated DNA sequencing (ddRAD-seq), permit high throughput discovery of sequence polymorphism (from thousands of markers spread across the genome) even without an existing reference genome (Peterson, Weber, Kay, Fisher, & Hoekstra, 2012). ddRAD-seq has been successfully used to genotype thousands of single nucleotide polymorphisms (SNPs) in multiple non-model organisms, which outperforms the traditional microsatellites approach (Andrews, Good, Miller, Luikart, & Hohenlohe, 2016). For example, studies have shown that by performing genome-wide SNP genotyping (ddRAD-seq) in wild populations of the deer mice (*Peromyscus*), the de novo analysis recovered 6,199 variable regions, and yielded 15,962 total polymorphic sites with genotypes for at least 70% of the 54 individuals studied (Peterson et al., 2012). Therefore, the genome exploration technique ddRAD-seq stands as a practical and informative method for comparing genomes of non-model organisms (de novo analysis without a reference genome) that can be used for genotype-phenotype association (GWAS).

Genome association studies (GWAS) measure the correlation between a DNA polymorphism and a trait (Visscher et al., 2017). These studies are popular nowadays with the advent of new genome exploration methodologies such as ddRAD-seq. Several studies have

shown that with the data generated by ddRAD-seq it is possible to determine if there are signatures of selection in the genome associated to a phenotype (Stephan, 2016). For example, GWAS in humans has shown that there are signatures of negative frequency-dependent selection for signaling individual identity (face morphology) (Sheehan & Nachman, 2014). Thus, GWAS by the means of ddRAD-seq constitute a promising methodology to determine selection signatures associated to complex traits such as individual recognition.

The northern wasp *Polistes fuscatus* and its reported geographic variation in individual recognition present an ideal opportunity to study the genetic basis of a complex social behavior through GWAS (chapter one). Here, we compare foundresses from different populations of *P. fuscatus* to determine the genetic basis of the geographic variation in individual recognition across a geographic transect of populations of *P. fuscatus*. To explore the genetic basis of the geographic variation in individual recognition, we performed GWAS on foundresses from the studied populations in chapter one. We then used ddRAD-seq from six populations of *P. fuscatus* that previously have been identified with the presence or absence of traits associated with individual recognition. We hypothesize that there is a genetic basis for the geographic variation in individual recognition exhibited in *P. fuscatus*. We predict that when comparing populations with and without the individual recognition trait, there will be loci under diversifying selection. Alternatively, if there are no signatures of selection, the results could suggest methodological and evolutionary constraints to detect loci under selection associated to the individual recognition trait.

METHODS

To explore the genetic basis of the geographic variation in individual recognition in *P. fuscatus*, we performed GWAS using six previously phenotyped populations of *P. fuscatus* (chapter one).

Individuals collected and used for the phenotyping experiments were also used in the genomic exploration. Table 3.1 summarizes the phenotypes expressed in each studied population. In brief, we analyzed the signalers' facial and abdominal in-population diversity to determine what populations have high or low color mark variations, a key trait in individual recognition. Additionally, we assessed the receivers' abilities to remember the individual identity of conspecifics. The per-population scored phenotypes (Table 3.1) were then used to generate phenotype-genotype associations.

Populations sampled and DNA extraction:

Foundresses ("queens") of *P. fuscatus* wasps were collected live in the last week of May in 2016, 2017, and 2018 following a transect composed by six populations from Roth Rock State Forest (Pennsylvania) to University of Michigan Edwin S. George Reserve (Michigan) in the USA (Figure 1.1 and Table 1.1 in chapter one).

A total of 59 individuals were used in the analysis, distributed in the six populations (table 4). To yield the molecular weight of the DNA extraction, we dissected the wasps' thoraces to extract the muscles inserted in this body tagma, then ground the tissue with a mortar and pestle. We then extracted and purified the DNA from the samples with DNeasy® Blood & Tissue kit (Qiagen, Valencia, CA, USA). DNA was quantified with Qubit® (Thermo Scientific).

Genomic dataset

The genomic library was prepared following the double-digest restriction site-associated DNA sequencing (ddRADseq) protocol of Peterson, Weber, Kay, Fisher, and Hoekstra (2012). Genomic DNA was digested using the restriction enzymes EcoRI and Msel (New England Bioloabs, Ipswich, MA). Digested products were then ligated to adaptors containing unique

barcodes using enzyme T4 DNA ligase (New England Bioloabs, Ipswich, MA). Size-selection of the individual barcode ligation products (350-450 bp) was performed using pippin prep (Sage Science, Beverly, USA). The fragments were amplified by eight cycles of PCR to incorporate the Illumina flowcell adaptor. Each step was followed by a clean-up with AMPure beads (using a 1.6× beads/DNA ratio). The library was sequenced in one lane of an Illumina HiSeq2500 to generate single-end 150bp reads at The Centre for Applied Genomics, Toronto, Canada.

To process the genomic data, we used the pipeline STACKS 1.35 (Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013) on a high-performance computing cluster (the Advanced Research Computing at the University of Michigan, Ann Arbor, MI, USA). PROCESS_RADTAGS program was used for filtering and demultiplexing of reads. To identify putative loci, we assembled the sequences de novo per individual with USTACKS program, using a minimum stack depth (m) of three reads and a maximum distance (M) of two different nucleotides between stacks. We built a catalogue of consensus loci with CSTACKS. Individual genotypes for all the loci in the catalogue were assessed with SSTACKS.

Individuals were assigned to their corresponding populations in the software POPULATIONS in stacks pipeline. In the population genetics analysis, we maximized the number of loci retained by using biallelic loci from a minimum of two populations. A custom script (available on https://github.com/KnowlesLab; Thomaz, Malabarba, & Knowles, 2017) was used in R 3.2.2 (R Core Team, 2017) to exclude loci with high theta values (located within the upper 95% quantile) and SNPs from the two last nucleotides to guard against sequencing and assembly errors. Following this step, the software PLINK 1.07 (Purcell et al., 2007) was used to identify SNPs with a maximum of 20% of missing data and with a minimum stack depth per individual (m) of five for inclusion in the final dataset. We then ran a PCA based on the SNPs

variance across the populations using the package Adegenet, R 3.2.2 (Jombart T. and Ahmed I., 2011).

In addition, to assess the correlation between geographic distances and genetic distances between the studied populations, we ran an isolation by distance analysis (Mantel test). As the measure of genetic distance between the populations, we used the Fst matrix (Table 3.2) and the geographic distance between the populations (Table 3.3). The analysis was run using the software IBD Version 1.52 (Bohonak, 2002). Finally, we used the software Tassel Version 5.2.51 to generate a neighbour joined cladogram (Bradbury et al., 2007). 14949 loci in 59 individuals spanning all six populations were included. The cladogram was unrooted.

Detection of loci under selection

To detect selection signatures associated with individual recognition in the *P. fuscatus* genome, we associated the phenotypes exhibited by each population (Table 3.1) and the loci retained using the software BayeScan Version 2.1 with the Fst – outlier approach (Foll & Gaggiotti, 2008). In BayeScan, selection is tested by decomposing Fst coefficients into a locus-specific component (alpha) shared by all the populations. When the locus-specific component is necessary to explain the observed pattern of diversity (alpha significantly different from 0), then departure from neutrality at a given locus is assumed. A positive value of alpha suggests diversifying selection, whereas negative values suggest balancing or purifying selection (Coulon, Deputte, Heyman, & Baudoin, 2009; Foll & Gaggiotti, 2008). Thus, BayeScan output shows the loci that are under directional selection (high Fst) and loci that are under purifying selection (low Fst) given the presence or absence of individual recognition trait across populations. We performed two phenotype/genotype associations. The first one was based on the signalers' facial and abdominal color mark diversity per population, and we wanted to know if there were

selection signatures (purifying or directional) in the genome of *P. fuscatus* associated with this diversity. Therefore, populations two and six were compared against populations one, three, and four. The second one was from the receivers' perspective, and we wanted to know if there were selection signatures in the genome associated with the ability to individually recognize conspecifics. Therefore, populations one, two, three, and four were compared against populations five and six.

Standard PLINK files were converted to the BayeScan format with the PGDSpider Version 2.1.1.5 software (Lischer & Excoffier, 2012). BayeScan analyses comprised 20 pilot runs with a length of each pilot run of 5000, a burn-in of 50000 iterations, a thinning interval of 10, with a resulting total number of 100,000 iterations.

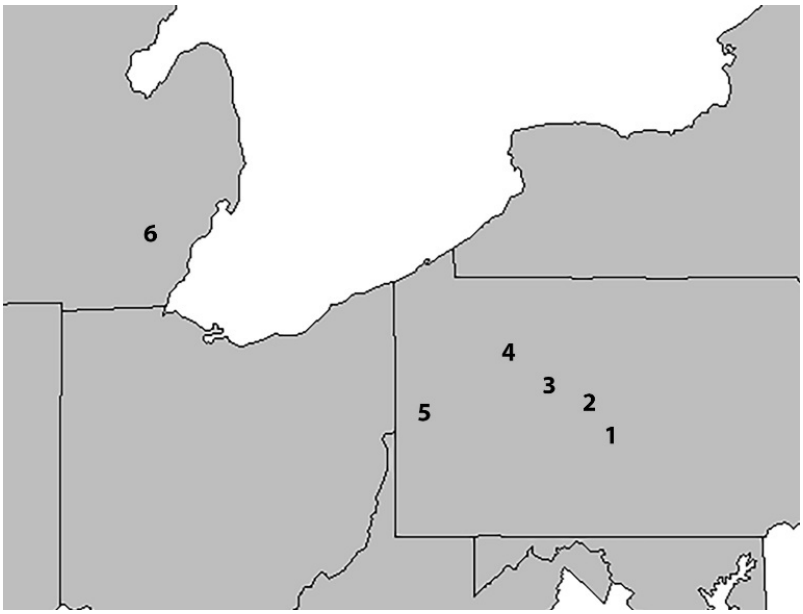

Figure 3.1. Distribution of sampling in the present study, from State College, Pennsylvania (1) to Pinckney, Michigan (6), USA. Each number represents a population. For the geographic coordinates refer to Table 1 in chapter one.

Table 3.1. Summary of the results for the phenotypes exhibited per population obtained in chapter one.

POPULATION	COLOR MARK VARIATION (signalers)	INDIVIDUAL RECOGNITION ABILITY (receivers)
1	low	not
2	high	not
3	low	not
4	low	not
5	poor data	yes
6	high	yes

RESULTS

(a) *Genetic diversity and genetic structure*

Over 136 million ddRAD-seq reads were generated for the 59 individuals in the six populations. After the bioinformatics process (stacks package), the number of loci that were consistent for all the individuals was 14949, which ended up being the polymorphic markers used for this analysis. The pairwise F_{ST} values (Table 3.2) showed that the genetic differences (allele frequencies) between populations are low, which suggests the existence of gene flow between the populations, supporting the same species status for all six populations. Commonly, Fst below 0.1 are considered as low genetic differentiation. For example, in human genetics, Fst below 0.1 are interpreted as lack of significant genetic structuring or population subdivision (Elhaik, 2012). Even though the cladogram showed that most of the individuals fall in groups according to their original population (Fig. 3.2), the PCA revealed that populations do not form clear clusters based on the genetic similarities; instead, they were mixed, and they could not be easily distinguished from each other, suggesting that the populations are inbreeding (Fig. 3.3). The PCA converted the 14949 observed SNP data into a set of values of linearly uncorrelated variables (PC1 and PC2) that summarize the variation between samples. Principal Component one explained 8.48% and Principal Component two explained 4.05% of the genetic variation between the populations. Although these percentages seem low, they fall in the common range reported in GWAS across

taxa (plants and animals) (Lenz, Muller, Zenke, & Schuppert, 2016). In addition, genome-wide heterozygosity was approximately equivalent across populations. Measures of genomic diversity (π) and H_{OBS}, H_{EXP}, indicated that the genomic diversity within populations was similar (table 3.4).

Moreover, when testing for isolation by distance, the Mantel test showed no correlation between geographic distances and genetic distances ($Z = 187.791$, $r = 0.1554$, one-sided $p = 0.438$ from 1000 randomizations), not supporting the idea that populations' genetic distances were explained by the geographic distances between them.

(b) *Outlier Single nucleotide polymorphisms*

When analyzing signatures of selection that are associated to the individual recognition trait in the *P. fuscatus* genome, the BayeScan results revealed that there were no candidate loci for selection associated with individual recognition based on the differences in allele frequencies between the populations. The posterior odds (PO) allowed the control of the False Discovery Rate (FDR), which is the expected proportion of false positives among outlier markers, which was set to 0.01. Figure 4 shows that none of the loci passed the FDR test for both approaches, signalers' color mark diversity, and for the receivers' individual recognition abilities. For the signalers' perspective approach, positive alpha values averaged 0.026 with a SD of 0.004, and negative alpha values averaged -0.057 with a SD of 0.063. From the receivers' perspective approach, positive alpha values averaged 0.005 with a SD of 0.003, and negative alpha values averaged -0.014 with a SD of 0.013. Jeffreys' interpretation on loci to be considered under selection completely rules out the alpha values found here for both approaches. Jeffreys' scale of evidence considers a value of alpha of 0.5 as barely worth mentioning, and a value of 1 to be decisive (Bradley, Gous, Kass, Datta, & Lahiri, 2001).

Table 3.2. Pairwise F_{ST} between populations of *P. fuscatus* based on the SNPs retained.

	2	**3**	**4**	**5**	**6**
1	0.049	0.06	0.063	0.036	0.058
2		0.05	0.052	0.055	0.051
3			0.079	0.068	0.074
4				0.068	0.072
5					0.06

Table 3.3. Geographic distances between the six populations in km.

	1	**2**	**3**	**4**	**5**	**6**
1	0	28.36	73.12	126	192.63	548.11
2		0	45.29	98.39	171.03	325.03
3			0	32.82	82.83	478.86
4				0	58.87	425.42
5					0	366.18
6						0

Table 3.4. Number of individuals sampled, N, and estimates of genetic diversity per population of *P. fuscatus* (see Figure 1 for distributional map of sampled populations).

Population	**N**	**H_{OBS}**	**H_{EXP}**	**π**
1	14	0.042	0.066	0.069
2	11	0.043	0.05	0.052
3	7	0.045	0.045	0.048
4	8	0.047	0.046	0.049
5	10	0.045	0.068	0.072
6	9	0.046	0.052	0.056

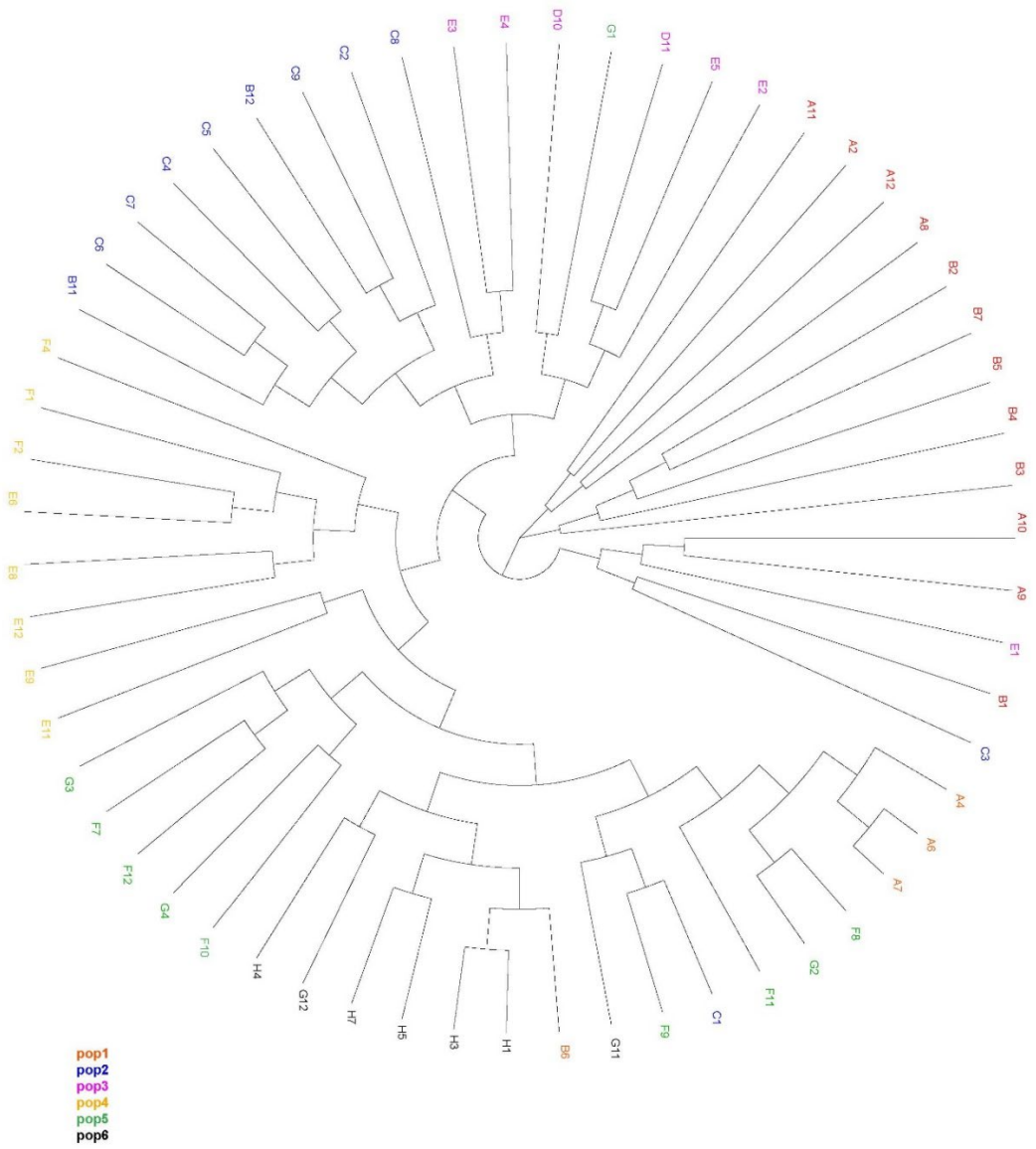

Figure 3.2. Cladogram including the individuals used in the study. The in-figure legend denotes by colors the population to which each individual belongs to.

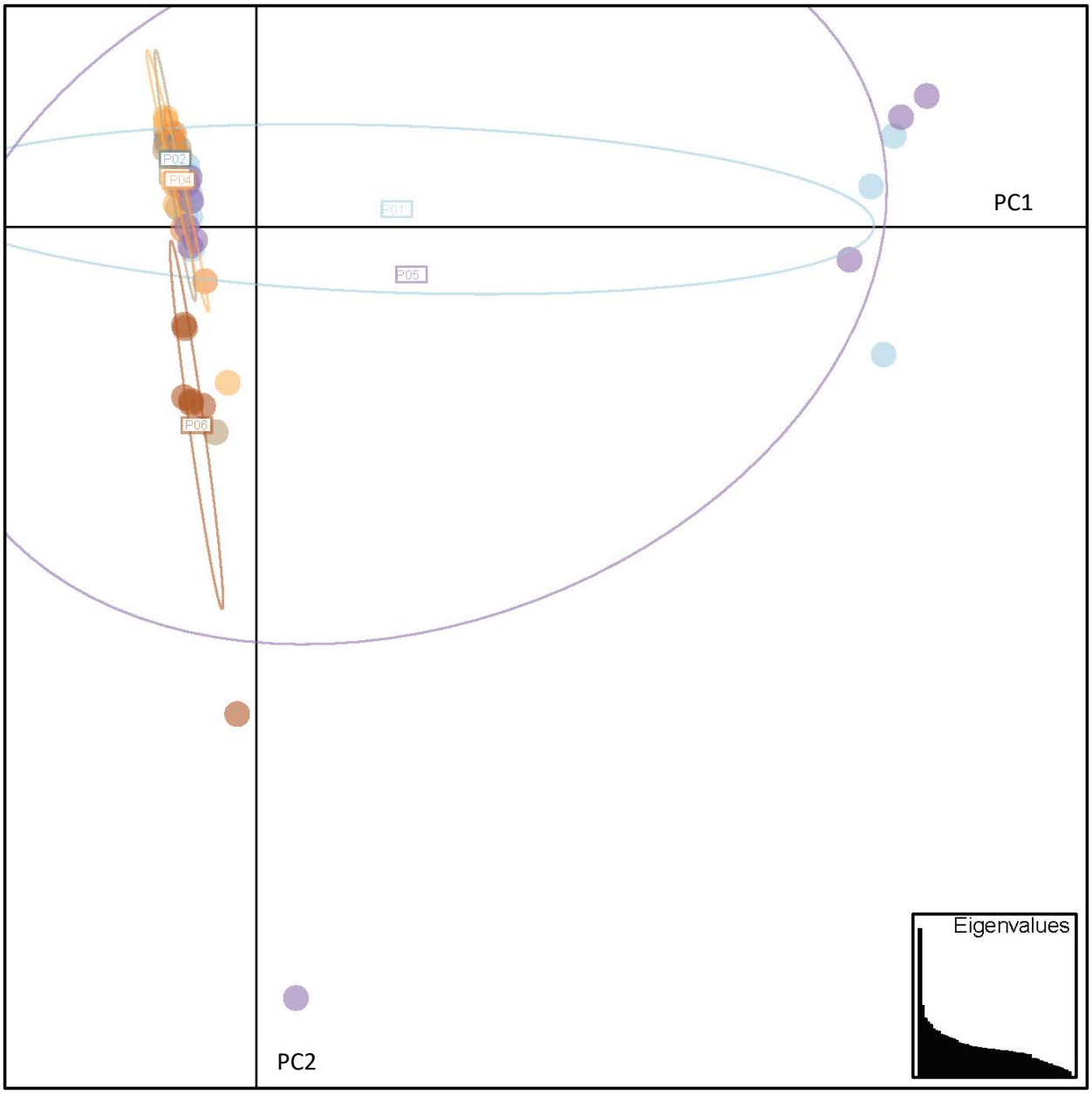

Figure 3.3. Principle Components Analysis (PCA) of *P. fuscatus* populations studied here. There are no clear clusters of populations, suggesting that the populations are not different species. Principal Component one explained 8.48% and Principal Component two 4.05% of the genetic variation.

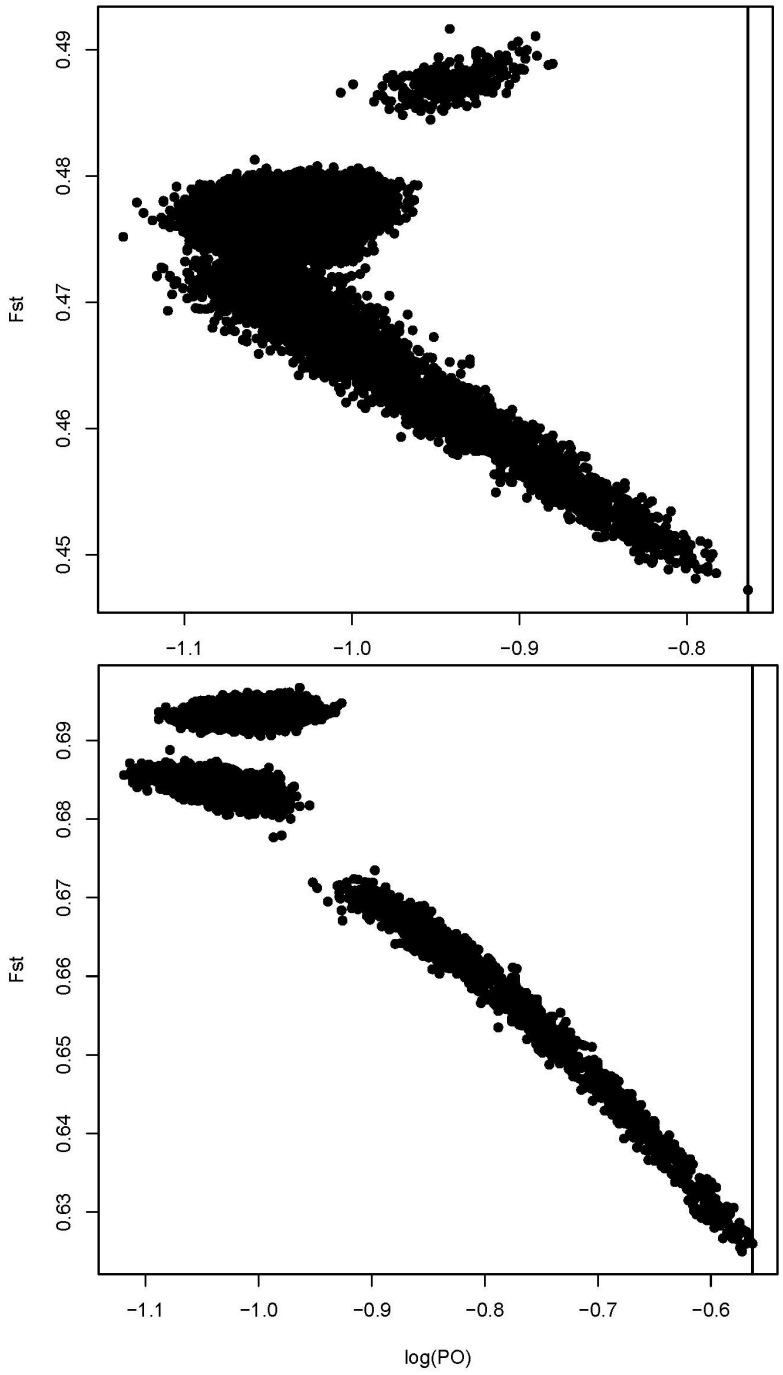

Figure 3.4. BayeScan plot of 14949 polymorphic amplified markers in the global genome scan analysis of 59 individuals from six *P. fuscatus* populations from Pennsylvania to Michigan, USA. F_{ST} is plotted against the log10 of the posterior odds (PO). The vertical line shows the critical PO used for identifying outlier markers. **A.** Analysis based on receivers' abilities to individually recognize conspecifics. **B.** Analysis based on the signallers' color mark variation.

DISCUSSION

We performed a Genome Wide Association Study using *P. fuscatus* genome and its natural variation in individual recognition behaviour, which was previously phenotyped in chapter one in six populations across a geographic transect. First, we found low levels of genetic differentiation at the genomic loci screened between the populations, suggesting panmixia. Second, when associating the loci screened with the phenotypes that allow for individual recognition to occur, the BayeScan analysis revealed that no loci exhibited a high degree of genetic differentiation across the populations. Thus, our approach did not find any signatures of selection on variable loci when associating the genome of *P. fuscatus* with the presence or absence of the individual recognition trait. These data together suggest that (i) there is gene flow between *P. fuscatus* populations, and (ii) our methodological approach to detect loci under selection was not appropriate, and *P. fuscatus* genetic architecture associated with individual recognition makes it difficult to find such genetic signature of selection for individual recognition trait.

(a) *Genetic diversity and genetic structure*

Although differences in behavior are expected to be important for reducing gene flow between populations, particularly when accompanied by ecological or morphological changes (Sattman & Cocroft, 2003; Smith, 1966), here, we found that even though there is variation in the presence of individual recognition across populations, the gene flow between the populations is maintained. The results suggest that individual recognition ability has not produced reproductive isolation and marked population structures. This is interesting because individual recognition is a social behavior that is involved in the configuration of the social hierarchies of *P. fuscatus* colonies (Tibbetts & Sheehan, 2013). That the populations have not genetically diverged even with

differences in social behaviors shows that populations of *P. fuscatus* are connected and gene flow might be swamping the genetic differences associated to the behavioral differences (Sexton, Hangartner, & Hoffmann, 2014). This finding is somewhat surprising as *P. fuscatus* foundresses are thought to be philopatric, nesting in the same natal nest the following year or very close to it (Klahn, 1979; West-Eberhard, 1969).

Highly mobile species are expected to show little spatial structuring at fine scales, while philopatric species are expected to show evidence of genetic population structure (Bose et al., 2017). Long term observations on *P. fuscatus* nests have shown that *P. fuscatus* foundresses are strikingly faithful to their natal nests (Klahn, 1979; Sheehan, Choo, & Tibbetts, 2017). Here, we did not make any measurements of foundresses' dispersal across populations. However, the genetic evidence presented here suggests that *P. fuscatus* achieve relatively high dispersals that interconnect the populations; however, how they maintain such interconnected populations is unknown. Future work should investigate the mechanisms that maintain the gene flow between the populations of *P. fuscatus* despite their philopatric behavior.

In *P. fuscatus*, individual recognition has been proposed as a mechanism that increases the phenotypic and genetic diversity of the population because rare phenotypes are favored via negative frequency-dependent selection (Sheehan & Tibbetts, 2009). These rare phenotypes are the unique identities of the signalers that make them recognizable as individuals (Tibbetts, 2002). Therefore, one could expect higher values of genetic diversity measurements in the populations that exhibit individual recognition than in populations that lack individual recognition. However, H_{OBS}, H_{EXP} and π values did not follow this pattern. For example, population six, which exhibits individual recognition, has lower measurements of genetic diversity than population one which lacks individual recognition (Table 4).

In humans, areas associated with signaling individual identity exhibit more genetic diversity than areas that are not associated with individual identity (Sheehan & Nachman, 2014); in *P. fuscatus*, this trend is not that clear. Previous work has shown that human faces have evolved to signal individual identity in the same way as *P. fuscatus*: via negative frequency-dependent selection. When comparing the genomic diversity of the areas associated with individual recognition in humans across several populations, a high genomic diversity (π) is shared across human populations, indicating that genomic diversity is consistently maintained across human populations to signal individual identity (Sheehan & Nachman, 2014). The measurements of genomic diversity in *P. fuscatus* across populations with and without individual recognition did not show extreme variations; rather, these measurements are equivalent between the populations (Table 3.4). However, these measurements of genomic diversity in *P. fuscatus* are from the whole nucleotide genome and not calculated for genome areas that are associated with individual recognition as was done in human studies. In spite of this, we can conclude that the negative frequency-dependent selection exerted on the *P. fuscatus* phenotypes does not affect the *P. fuscatus* genome as a whole; otherwise, the genome diversity between the populations with and without individual recognition would present considerable differences. Future studies on the genomic diversity in areas associated with individual recognition in *P. fuscatus* are needed to determine the impact that negative frequency-dependent selection has on the *P. fuscatus* genome diversity.

(b) *Outlier Single nucleotide polymorphisms*

There was no evidence of signatures of natural selection in the *P. fuscatus* genome associated with individual recognition across the studied populations. There are three explanations on why we failed at finding loci under selection associated with individual recognition. First, the

ddRAD-seq approach may not be suitable for this study. Recent research on genome scans based on ddRAD-seq data alone have shown that, while useful for assessing neutral genetic variation and genetic population structure, this method will probably miss a substantial amount of loci under selection when exploring for local adaptation (Lowry et al., 2017). Because of the relatively low density of markers produced, ddRAD-seq marker data sets are often too separated to have a reasonable chance for approaches such as BayeScan of detecting the loci involved in adaptation (Lowry et al., 2017) . Despite the novelty of ddRAD-seq, there is empirical evidence where GWAS failed in identifying candidate SNPs that were previously identified *in silico*, even in a model organism. In fact, the results showed that only 30–70% of the top 20 *in silico* array candidates were within 1 kb of sequence-based (ddRAD-seq) candidates (Stanton-Geddes et al., 2013). Thus, marker sparseness is an issue for GWAS.

Despite ddRAD-seq issues with the representability of the actual studied genome, ddRAD-seq can be very useful for estimating parameters that carry information on how adaptation occurs, such as population structure and gene flow (Andrews et al., 2016; Lowry et al., 2017; Peterson et al., 2012). Other studies have found ddRAD-seq useful for genomic scans of local adaptation when candidate genes have been previously identified (Lowry et al., 2017; Natarajan et al., 2015). Genome scans based on ddRAD-seq data alone will inherently carry the issue of missing many loci involved in local adaptation, just by chance (e.g. genome fragments obtained).

Second, signatures of selection for individual recognition trait were not detected by BayeScan because individual recognition might be an evolutionarily recent trait with fast polygenic adaptation. Theoretical and empirical approaches have shown that BayeScan-like methods fail to detect significant frequency shifts for individual SNPs between populations that are suffering rapid adaptation at multiple loci (polygenic adaptation) (Jain & Stephan, 2017;

Stephan, 2016). Previous work in individual recognition in *P. fuscatus* has shown that the facial color marks that signal individual identity exhibit genetic heritability, suggesting that multiple loci are underlying the facial color pattern variation present in signalers of *P. fuscatus* (Sheehan et al., 2017). Moreover, *P. fuscatus*' individual recognition could be a trait under rapid adaptation. *P. fuscatus* is the only Polistine tested so far that exhibit individual recognition. Therefore, a whole-genome-study stands as a more suitable approach to find fine-scale differences in GWAS where there is high potential for fast polygenic adaptation.

And third, phenotypic plasticity can produce different phenotypes across populations without underlying genetic differentiation (Foster, 2013). Thus, different phenotypes can be produced by a single genotype, under different environmental conditions. Although we did not test directly for phenotypic plasticity in the present study (we would need a different experimental approach), our results give insights on the possible behavioral plasticity in *P. fuscatus*. Under high levels of gene flow, plasticity is expected to occur, because high gene flow constrains the possibility for local adaptation, resulting in similar genotypes with the ability to express several phenotypes (Scheiner, Barfield, & Holt, 2012; Sexton et al., 2014; Sultan & Spencer, 2002). Here, we reported a low genetic differentiation between *P. fuscatus* populations, which is evidence of gene flow. The presence or absence of individual recognition in the studied populations could be the result of phenotypic plasticity via strong gene flow between the populations. However, at least from the signalers' perspective in *P. fuscatus*, the individual identity marks are highly heritable, which suggests that individual identities have a genetic load that determines the phenotype expression (Sheehan et al., 2017). Therefore, directed experiments to test for phenotypic plasticity in *P. fuscatus* are needed to determine the nature of the variation of individual recognition.

Developmental plasticity in face learning has been reported in phylogenetically closely related species to *P. fuscatus*. *Polistes metricus*, a paper wasp that lacks individual recognition ability, shows improvement in facial color mark learning when reared with *P. fuscatus* wasps (Tibbetts, Pandit, & Nondorf, 2018). This finding is relevant because it shows that the ability to learn unique faces has strong developmental plasticity properties that could be adding to the geographic variation found in *P. fuscatus*' individual recognition. Thus, the geographic variation in individual recognition in *P. fuscatus* could be a very complex trait, with genetic and ontogenetic basis. Future studies should aim to disentangle the factors associated with such a variation.

Overall, we found that even though there is geographic variation in individual recognition across populations of *P. fuscatus*, we did not find genetic signatures showing that individual recognition is under natural selection in the studied populations. Gene flow between the studied populations is present, which could be swamping the evidence that some loci are under natural selection when comparing the populations' genomes under the light of the individual recognition phenotype. The methodological approach used here, ddRAD-seq, could explain also in part why selective sweeps were not found. ddRAD-seq is a reduced-genome approach, so just fragments of the genome are obtained, leaving a low chance for BayeScan to detect loci under selection. Besides, phenotypic and developmental plasticity in individual recognition might be adding variation in the expressed phenotypes in the populations studied. In conclusion, fine-scale genomic differences to detect selection on complex behaviors require, if possible, an assessment of the whole-genome of the studied organism. In addition, developmental and phenotypic plasticity might play a role in the observed variation in *P. fuscatus*' individual recognition.

Future studies directed to test for plasticity in individual recognition should be conducted using whole-genome data.

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CHAPTER FOUR

SOCIAL CONTEXTS AND COMPLEX SIGNALS INTEGRATION: INDIVIDUAL AND CLASS-LEVEL RECOGNITION IN THE PAPER WASP *Polistes fuscatus*

ABSTRACT

Most social animals communicate through complex signals, yet we do not know how social contexts affect the production and integration of complex signals. Understanding complex signals in social contexts is important because what could be a key communication form in one taxa could be detrimental in other, thus we can better comprehend the evolution of communication in social species. The eusocial wasp *Polistes fuscatus* exhibit two forms of recognition: individual and class-level recognition, however we do not know how the signals associated to each type of recognition interact between them and how they are integrated in the receivers. Previous studies on *P. fuscatus* have shown that visual signals are important for individual recognition, and that chemical signals are important in class-level recognition. Here, we experimentally manipulated visual and chemical signals in workers of *P. fuscatus* to determine what signal will nest-members prefer when performing nest-member recognition (e.g. class-level recognition). We found that workers of *P. fuscatus* prefer chemical signals (cuticular hydrocarbons) over visual signals (face and abdominal color marks) for nest-member recognition. These data suggest that *P. fuscatus* integrates the information from multimodal signals according to certain social requirements. When comparing these results with other social taxa that exhibit complex signals for recognition, we conclude that

social needs in social species are responsible for elaborated multimodal signals interaction, but not a matter of nervous system complexity.

INTRODUCTION

Many animals communicate using complex signals that convey information in multiple sensory modalities. Complex signals are typically divided into two main categories based on information content of the signals: redundant signals convey similar information, while non-redundant signals (or 'multiple messages') convey different information (Hebets & Papaj, 2005). For example, wolf spiders have redundant sexual signals, as female wolf spiders assess the quality of potential male mates via both visual signals (male leg bristle size) and vibratory signals (amplitude and peak frequency of leg strikes) (Stoffer & Uetz, 2017). In contrast, Eland antelope have non-redundant sexual signals, as males signal body size via the frequency of knee clicks and age via dewlap size (Bro-Jørgensen & Dabelsteen, 2008). Thus, a key point in the understanding of complex signals' functionalities is to determine the information conveyed by the signaler and the response elicited by the receiver.

Although categorizing signals based on information content has been valuable, it is also incomplete. The way researchers measure and define 'information' will influence the categorization such that two researchers using different methods could potentially categorize the signal in different ways. Instead of relying on researcher signal design, ask the receiver what matters (Rendall, Owren, & Ryan, 2009). As a result, there is a push toward testing receiver responses complex signals. In particular, testing receiver responses to each signal in isolation as well as the composite signal provides crucial information about how receivers use and integrate information from complex signals (Partan & Marler, 2005).

Testing how receivers integrate information from complex signals is important because receivers often respond to complex signals in unexpected ways. In some cases, receiver responses are context dependent, such that receivers respond to one signal in some situations and a different signal in other situations. In other cases, one signal is 'dominant', so receivers pay more attention to one signal than the other signal across contexts (Higham & Hebets, 2013). Complex signals can have interactive effects that produce unexpected patterns of receiver responses. For example, receivers may respond differently to the combination of two signals than to either signal in isolation. In *Anolis* lizards, signalers are detected easier when combining bright colors and movements (raising and lowering) of the dewlap (Fleishman, 2000). Contrastingly, in the garter snakes, *Thamnophis ordinoide*, the combination of striped patterns and antipredator evasive behaviors such as reversals (stereotypical changes in direction) make the snake less detectable to receivers (Brodie, 1992).

Thus far, much of the work on how receivers respond to complex signals has focused on signals used during mate choice, maybe because the elaborated mating signals found in nature. For example, the striking bill and plumage coloration of the American goldfinch (*Carduelis tristis*) are estimators of overall quality used by the females for mate choice (McGraw & Hill, 2000) (Candolin, 2002). However, growing research on non-sexual complex signals has shown the importance of complex signals in the maintenance of social interactions not associated with mating. For example, in *Apis mellifera* at least three sensory modalities are known to be involved in spatial information about floral resources (Dyer, 2002). There is a rich field on non-sexual complex signals that has not been explored.

Taxa with individual identity signals provide a particularly interesting perspective on complex signaling because individual identity signals can be linked with multiple types of

information. As a result, individual identity signals could interact with other signal types to influence receiver responses in unexpected ways. During individual recognition, receivers learn the unique characteristics of signalers, associate the signaler's phenotype with information about the signaler, then recall the phenotype-information link during subsequent interactions. The information receivers associate with individual identity signals varies and includes traits like: kinship, group membership, dominance rank, previous history of social interactions, or territory location (Cely & Tibbetts, 2017; Tibbetts & Dale, 2007).

Receivers link individual identity signals with many different types of information, so there may be interesting interactions between individual identity signals and other signal types. For example, some species use individual identity signals to assess dominance rank of familiar individuals and also have status signals that provide information about fighting ability (Hebets & Papaj, 2005). Receivers could: a) use individual identity and status signals in different contexts, b) pay more attention to one signal than the other signal, or c) integrate information about fighting ability from both individual identity and status signals. Thus far, we know little about how receivers integrate information from individual identity signals and other signal types. For example, some species use individual identity signals to assess dominance rank of known individuals and they also have separate signals that provide information about group membership. Receivers could use individual identity and group membership signals in different contexts; group membership signals to determine whether to allow an individual into the group and individual identity signals to assess dominance rank of group members. Alternatively, they could integrate information from both signals. For example, in zebra finches, redder beaks and higher song rates in males can be integrated to assess for the same information: high quality

males (Birkhead et al. 1998). Thus far, we know little about how receivers integrate information from individual identity signals and other signal types.

The paper wasp *Polistes fuscatus* (Fabricius, 1793) is a good model to study how individual identity signals interact with other signal types. *P. fuscatus* have variable facial patterns that function as visual signals of individual identity (Tibbetts, 2002). Conspecifics learn and remember the unique phenotypes of conspecifics during social interactions. Receivers use individual identity signals to keep track of dominance relationships with conspecifics, and individual recognition reduces aggression and stabilizes dominance hierarchies (Tibbetts, 2004). *P. fuscatus* also have chemical signals of nestmate identity. Wasps from the same nest have similar cuticular hydrocarbon (CHC) profiles (Espelie, Gamboa, Grudzien, & Bura, 1994; Gamboa, Grudzien, Espelie, & Bura, 1996) and wasps with non-nestmate CHC profiles are attacked if they land on a nest.

Here, we test how visual signals of individual identity and chemical signals of nestmate identity interact during social assessment in *P. fuscatus* wasps. Paper wasps learn the unique facial patterns of each nestmate, so they could use facial patterns to identify nestmates vs. non-nestmates. Alternatively, they could rely on CHCs alone for nestmate recognition. We experimentally manipulated visual and chemical signals using a factorial design to test how receivers respond to models with the following traits: A) facial patterns and cuticular hydrocarbons of nestmates, B) Facial patterns of nestmates and cuticular hydrocarbons of non-nestmates, C) facial patterns of non-nestmates and CHCs of nestmates and D) facial patterns and CHCs of non-nestmates. If wasps use chemical signals alone to identity nestmates, they will be more aggressive toward models with non-nestmate CHCs, while facial patterns will not influence aggression. If wasps use both CHCs and visual signals to identify nestmates, both non-nestmate

CHCs and facial patterns will increase aggression. Visual and chemical signals could interact such that models with both non-nestmate CHCs and facial patterns will receive more aggression than other models. Alternatively, there could be no interaction between visual and chemical signals such that models with either CHCs or facial patterns of non-nestmates will receive similar amounts of aggression.

METHODS

Overview of methods:

Polistes fuscatus (Fabricius, 1973) used in this experiment were from two locations separated by 87 km. in Southeast Michigan, USA: The University of Michigan George Reserve (GR) 42.458681, -84.011462 (274 masl) and The University of Michigan Matthaei Botanical Gardens (MBG) 42.301226, -83.663073 (243 masl). The experiments were carried out in July and August of 2016 and 2017. We tested the behavioral response of wasps on each nest to four types of lures: wasps with a) the facial pattern and cuticular hydrocarbons of nestmates, b) the facial patterns of non-nestmates and cuticular hydrocarbons of nestmates c) the facial patterns and cuticular hydrocarbons of non-nestmates, or d) the facial patterns of nestmates and cuticular hydrocarbons of non-nestmates (Table 4.1). We recorded nests during lure presentation and later measured aggression toward the lures.

Lure preparation:

Lures used in the experiment were fresh-killed wasps collected the night before the behavioral assay. Two workers were collected from each nest to be used as lures. We followed the methods of Baracchi *et al* (2015) to create lures.

We manipulated the chemical signals of the lures by removing, then reapplying the CHCs from each wasp. Pentane removes the CHCs from a wasp, allowing us to transfer CHCs from one wasp to another. Immediately after collection, each wasp was placed in new glass vials with 1 ml of pentane for ten minutes. The 1 mL of pentane and CHCs was dried overnight at room temperature, resuspended in 200µL of pentane and then split in two aliquots. The aliquots were then placed on the head, thorax, abdomen, and wings of lures that had previously been washed with pentane to remove CHCs. Each of the aliquots were applied to different lures. In some cases, the CHCs were re-applied to the same wasp, creating lures with nestmate faces and nestmate CHCs or non-nestmate faces and non-nestmate CHCs. In other cases, the CHCs were applied to different wasps, creating lures with mis-matched faces and CHCs.

Each lure was used twice on two different nests. Lure order was randomized across trials to ensure order did not alter results. For example, a lure with facial patterns and CHCs from nest A would be used as a nestmate lure for nest A and a non-nestmate lure for nest B. Similarly, a lure with facial patterns from nest A and CHCs from nest B, would be used as a lure with nestmate faces and non-nestmate CHCs for nest A and a lure with non-nestmate face and nestmate CHCs for nest B.

After altering the CHCs, wasps were attached to a 50 cm. metal stick by an entomological pin inserted through the wasp's thorax. Antennae, head and wings were positioned to ensure the lure looked as similar as possible to a live wasp. Wasps responded to lures as if they were live wasps (Forsyth, 1975; Gamboa, Wacker, Duffy, Dobson, & Fishwild, 1992).

Table 4.1. Factorial design for the lure types presented to each nest.

Lure type	Face	CHC profile
A	Nestmate	Nestmate
B	Alien	Nestmate
C	Alien	Alien
D	Nestmate	Alien

Lure presentation:

We presented four lures sequentially to a focal nest and scored aggression toward each lure. The four lures were presented in a pseudo-random order. During presentation, the lure was moved slowly toward the nest, then held stationary at 2 cm from the nest for 30 seconds. Wasps responded naturally to the lure, attacking non-nestmate lures with a similar amount and intensity of aggression as typically seen when non-nestmates land on a nest (West-eberhard, 1986). Lure presentation was video-recorded for later behavioral analyses. The experimenter was blind to lure identity during presentation.

Aggression toward the lures was scored by counting how frequently nestmates bit, mounted, and partially mounted the lure. Define each behavior These aggressive behaviors in *P. fuscatus* are stereotyped dominant behaviors, which have been traditionally used for assessing for aggression (West-Eberhard, 1969). Behavioral trials were scored by only one observer blind to the experimental predictions.

Statistical analysis

Data were analyzed using a linear mixed model in SPSS version 24. The dependent variable was the number of aggressive acts directed toward the lure (log x +1) transformed. Fixed effects were lure type (categorical, A, B, C, or D, table 1) and the order of lure presentation (continuous, 1-4). Focal nest identity and the particular lure identity were included as a random effect. Year was not included as an effect in the model because aggression did not vary across years. Pairwise post-hoc analyses to compare aggression between the four lure types were performed using a LSD test. In total, 60 lures were created from 17 nests. 96 trials were run on 17 nests.

RESULTS

Aggression was influenced by lure type ($F = 6.216$, $DF = 3$, $n = 96$, $p = 0.001$). Post-hoc analysis shows wasps were most aggressive toward lures with alien CHCs. Lures with alien CHCs received more aggression than lures with nestmates CHCs. However, having alien faces did not influence aggression, as lures with alien faces received no more aggression as lures with nestmates faces (Fig. 4.1 and Table 4.2).

Table 4.2. Summary of the p-values for the LSD post-hoc comparisons between the lures. Asterisks denote statistical significance to the level of < 0.05.

	B	**C**	**D**
A	0.19	<0.0001*	0.001*
B		0.019*	0.032*
C			0.859

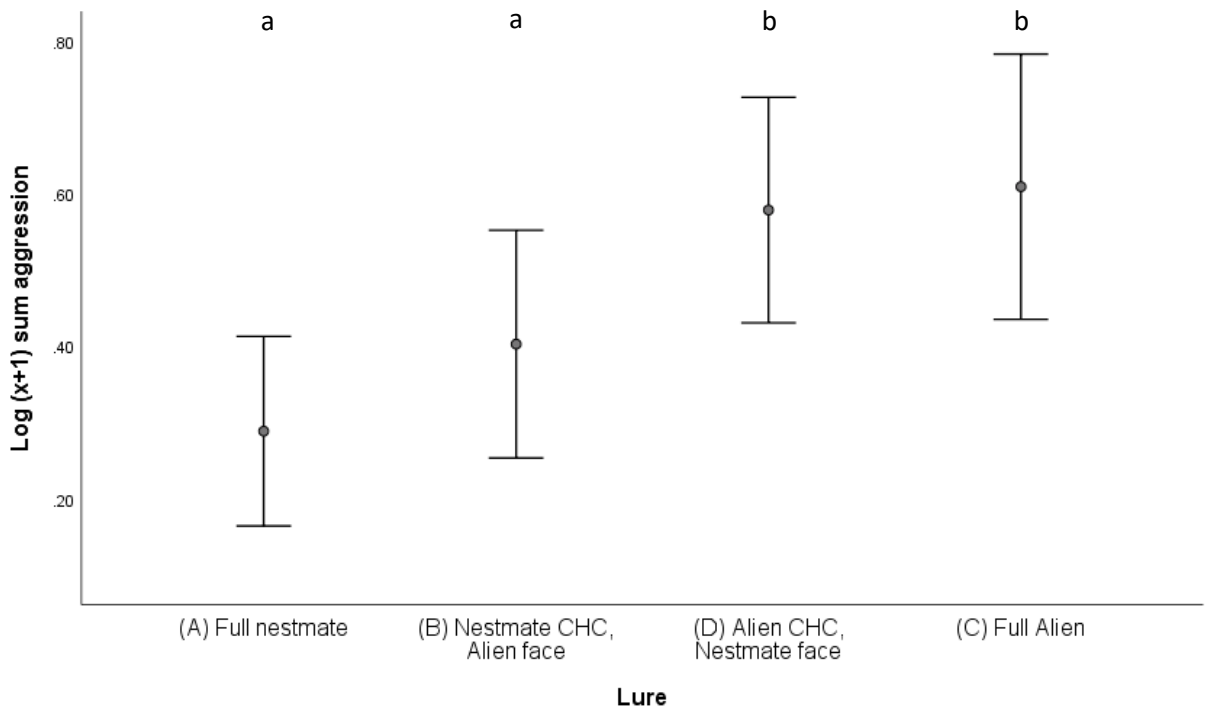

Figure 4.1. Means of aggressive behavior (Log transformed aggression index) toward each of the lures. Whiskers denote ±2 SE. Different letters on top of the boxes represent significant differences.

DISCUSSION

P. fuscatus prefer chemical signals over visual signals for immediate recognition of nest-members. Visual signals (facial and abdominal marks) and chemical signals (CHCs profile) convey different information in *P. fuscatus*. While chemical signals provide information regarding nest-membership and are used for class-level recognition, visual signals provide information about individual identity and are used for individual recognition.

Instead of using only one signal for recognizing conspecifics, individual recognition and class-level recognition in *P. fuscatus* are mediated by two different signals in two different sensory modalities. But what is more interesting is that the preference of one signal over the

other is dictated by the social context. When the social context requires the assessment of group membership, *P. fuscatus* process information coming from chemical signals but when the social context demands individual recognition, *P. fuscatus* process information coming from visual signals. This finding is interesting because thus far we do not understand how individual identity signals interact with other type of signals in social contexts.

Individual identity signals are special because they provide a wide range of information about the signaler such as quality, social status, and territory, while class-level recognition signals provide specific information regarding only group membership (Tibbetts & Dale, 2007). For example, one can think that by individually recognizing a conspecific, the receiver by extension can assess other more general characteristics such as group-membership than merely the individual identity of the signaler. For instance, the white throated sparrow (*Zonotrichia albicollis*) individually recognizes its neighbors by hearing their songs and uses this information for recognizing territorial boundaries (Stoddard 1996). Why *P. fuscatus* are using two different signals for recognition of conspecifics when the information needed for individual and class-level recognition could be potentially be transmitted using only individual identity signals?

From the signaler perspective

Picture a *P. fuscatus* worker landing on the nest. nest-member recognition must be performed fast to avoid usurpers or exploiters, but at the same time it should be accurate, then efficient signals are expected. CHCs in *P. fuscatus* can be an efficient vehicle for conveying information regarding class-level recognition because: i) CHCs are organic molecules with the longest carbon chains made of around 40 Carbons and no less than around 20 Carbons (Espelie et al., 1994). This chemical property makes CHCs stable molecules with very low volatility, which allow the receivers to have an accurate perception of the signaler CHC profile without "cross-

contamination". ii) CHCs are covering the cuticular surface of the animal. It is only needed to perform antennation on almost any part of the body to get access to the information carried in the CHC profile (Singer, 1998). And iii) All members of the nest carry the same CHC profile. Thus, the amount and type of information carried is the same for everyone in the same nest, which makes easier for the receiver to discriminate between nestmates and non-nestmates (Espelie et al., 1994). These characteristics make CHCs an efficient signal with enough information for nest-member recognition.

On the other side, performing individual recognition relies more in the quality and amount of information conveyed than the efficacy of the signal. Individual recognition is particularly important in the founding phase of the nest. When *P. fuscatus* wasps begin their nests in spring, the foundresses engage in agonistic dominant interactions to stablish reproductive control of the nest (Reeve H., 1991). Hierarchies are formed by assessing signals of individual identity, once a dominant interaction is settled there is no need for further costly fights (Tibbetts, 2004). These signals of individual identity are very variable color marks in their faces and abdomens (Tibbetts, 2002). Each individual has a particular set of facial and abdominal marks that makes that individual unique. *P. fuscatus*' individual recognition requires a complete visualization of the signaler's head and abdomen. In fact, foundresses can engage in agonistic interactions for hours, while nest-member recognition must be performed in seconds. In this social context, the content is more important than the propagation. The information conveyed is not binomial as in group-membership recognition, in this case visual signals carry a set of information of individual identity that the receiver can use for recalling previous encounters outputs, a complex task that requires a memory associated to each individual in the nest (Sheehan & Tibbetts, 2008). The combination of visual marks in the face and abdomen in each

wasp carries more information than a same chemical profile shared by all the nest members as happens in nest-member recognition. This trade-off between content and efficacy for individual and nest-member recognition could explain why signalers use two different modalities for signaling identity to conspecifics.

From the receiver perspective

Performing individual recognition is more cognitively demanding than performing nest-member recognition, which could explain why *P. fuscatus* use two separate signals when individual identity signals could potentially be used in both types of recognition. In individual recognition, the receiver behaves in a unique way toward the signaler by matching the individual-identity characteristics of the signaler with information acquired in previous encounters (Cely & Tibbetts, 2017). Thus, this learning process requires the processing of large amounts of information (individual variation in facial and abdominal marks) and recalling precise information about particular previous encounters (memory). In fact, individual recognition is considered the most complex form of recognition (Tibbetts & Sheehan, 2013). Contrarily to individual recognition, class-level recognition in eusocial insects has been explained by self-organization rules that do not require learning and memory. For example, experiments on foraging with ants (*Linephitema humile*), where two equally large trails connected independently to a food source are presented has shown that one trail is randomly selected and posteriorly fixed via pheromones as the foraging trail (Bonabeau, Theraulaz, Deneubourg, Aron, & Camazine, 1997). Similarly, the decision on one CHC profile over the others in *P. fuscatus* can be the product of an early nest stage decision on a CHC profile, that posteriorly is fixed by repetition as the nest signature, which can be amplified given the binary nature of nest-member recognition (e.g. yes or not).

Then, performing individual recognition for social contexts where class-level recognition is needed is economically expensive.

Note that here we are not dealing with the evolution of this phenomena but with the proximate mechanisms that make possible individual and class-level recognition in *P. fuscatus*. The evolution of complex signals for recognition (from the signaler and receiver perspectives) is an open question in eusocial insects.

Complex signaling for recognition in other social animals

Social insects that do not require individual recognition use complex signaling in a different way than *P. fuscatus*. Pioneering research on the primitive eusocial wasp *Liostenogaster flavolineata* has shown how visual and chemical signals work as redundant signals for nestmate recognition, and how they are integrated to increase the accuracy and speed of the receivers' response (Baracchi, Petrocelli, Chittka, Ricciardi, & Turillazzi, 2015). When a conspecific arrives to the nest, the members of the nest assess nest-membership via chemical signals (e.g. CHC profile) and visuals signals (e.g. facial marks). In this case, *L. flavolienata* prioritizes visual signals (face marks) for nest mate recognition and later the use of chemical signals as an 'err on the safe side'. Both signals carry the same information and trigger the same behavior in the receiver (clas-level recognition), opposite than in *P. fuscatus*, where each signal carries different information.

Why *L. flavolineata* and *P. fuscatus* have different multi-sensory integration mechanisms? The answer may lay in the differences of recognition abilities and their life histories between the two species. *L. flavolineata* can discriminate familiar from unfamiliar faces, but they do not exhibit individual recognition as in *P. fuscatus.* In fact, the facial marks patterns in *L. flavolineata* covaries strongly with the CHCs profiles, suggesting a correlation of

facial markings with nest membership (Baracchi, Turillazzi, & Chittka, 2016). Further, *L. flavolineata* live in aggregations where several nests are close together and the social groups hierarchies are settled by gerontocracy instead of agonistic dominance as occurs in *P. fuscatus* (Bridge & Field, 2007). *L. flavolineata* may use redundant signals because they need more accuracy when recognizing a nest-member given their very close proximity to other nests, but more importantly because they do not need to memorize individual identities given their gerontocracy hierarchical system which does not require costly fights for settling down the winner.

In contrast to *P. fuscatus*, other social taxa such and in non-human primates, different messages regarding individual identity and group-membership can be simultaneously integrated and transmitted using only one modality. In a study using only playbacks in baboons, the authors found that baboons can determine kinship (class-level recognition) and at the same time the individual social rank of the signaler (individual recognition) (Bergman, Beehner, Cheney, & Seyfarth, 2003). In this case, baboons can use information coming from individual identity to assess other type of information such as kinship. This contrast tells us that extracting more general information from individual identity signals is possible, and that particular pressures in each social species can select for different types of signals integration. Baboons and *P. fuscatus* life histories and social contexts are very different despite their social organization, what is economically expensive for *P. fuscatus* might be a key trait in baboons.

In addition, other non-human primates can simultaneously integrate multimodal signals for discriminating between familiar and unfamiliar monkeys, feature that is not present in *P. fuscatus*. When presented visual and acoustic stimuli, Rhesus macaques' faces and vocalizations seem to be undividable representations of individuals. In human infants, this undividable

representation appears before the ability of sequentially match a voice with a face (Habbershon, Ahmed, & Cohen, 2013). In contrast, *P. fuscatus* use facial and chemical signals separately. There is no a simultaneous integration of these two signals. However, one can think that this ability of multimodal integration can be performed only in taxa with "complex brains", but as described above, the primitive eusocial wasp *L. flavolineata* can perform such a complex task for nest-member recognition. Therefore, the constraints for this type of multimodal integration might be not a "brain size" issue but a social need.

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CONCLUSIONS

In this dissertation, we have studied the intraspecific variation in the recognition system across geographically distant populations and social contexts in the eusocial wasp *Polistes fuscatus*. In chapter one, we analyzed the natural variation in the senders' phenotypes and receivers' learning abilities for recognizing conspecifics. In chapter two, we explored the genetic basis of individual recognition. Finally, in chapter three, we were interested in knowing how *P. fuscatus* wasps integrate multimodal signals when recognizing a nestmate. This work provides a body of empirical data supporting the idea that complex social behaviors are not species-typical; instead, they can be very flexible intraspecifically. Thus, intraspecific variation in social behaviors might be an underappreciated factor impacting the phenotypic and genetic diversity in social animals. We contributed to the following aspects in the advancement of the understanding on the evolution and maintenance of recognition systems.

1. *Social behaviors are not set for an entire species; instead, they show intraspecific variation*. Intraspecific variation of behaviors is a relatively recent area of research (Foster, 2013). In the past, behaviors exhibited in a species were expected to be geographically uniform (Foster and Endler, 1999). However, behavioral ecologists have come to assume that behavioral evolution can be adaptive, resembling patterns of variation shown in other, more studied traits such as morphological and molecular traits

(Foster, 2013). Here, we showed that behaviors such as individual recognition exhibit striking variation, demonstrating geographical variation in morphological traits.

The causes of geographic variation in *P. fuscatus* individual recognition remain to be uncovered. Although we tried to give a complete description of how individual recognition in signalers and receivers varies through showing the morphological and behavioral differences between populations, we did not provide an answer to why these phenotypic differences are present in *P. fuscatus*. A possible explanation is that social requirements in *P. fuscatus* are not the same across the studied populations. When *P. fuscatus* foundresses start the founding nest stage, foundresses interact with each other through agonistic dominance behaviors to control the colony reproduction (West-Eberhard, 1969)(Reeve, 1991). Individual recognition is thought to reduce these aggressive interactions (Tibbetts, 2002). However, *P. fuscatus* wasps from populations that do not exhibit individual recognition could form their nest predominantly alone, therefore avoiding contests for reproductive control of the colony; thus, individual recognition is not required. Future work describing the foundation process in nests that lack individual recognition should be conducted to determine if early social interactions drive the expression of individual recognition in *P. fuscatus*.

2. *Behavioral flexibility can be maintained via gene flow*. A large body of literature has shown how morphological and molecular traits are affected by gene flow, but how behavioral traits are affected by gene flow has received less attention (Thompson, 1999) (Pinho & Hey, 2010). Here, we showed that gene flow between populations may swamp

the signatures of local adaptation for complex behaviors such as individual recognition. It is thought that when gene flow between populations is high, little adaptive differentiation is expected because gene flow blurs the boundaries between populations (Thompson, 1999). Our results presented here support this idea. Strong gene flow could then lead to the situation where different phenotypes can be produced by a single genotype under different environmental conditions (West-eberhard, 2003); (Foster, 2013). Future research should perform directed experiments that test for phenotypic plasticity in individual recognition in *P. fuscatus*.

3. *Genome reduced-representation approaches inherently carry the issue of missing many loci involved in local adaptation, making it difficult to detect loci under selection associated with polygenic complex traits such as individual recognition*. ddRAD-seq marker data sets are often too separated to have a reasonable chance for approaches such as BayeScan to detect the loci involved in adaptation (Lowry et al., 2017). It is likely that there are loci under selection associated with individual recognition, but the methodological approach did not detect them. This methodological constraint limits analysis of the whole genome. Although ddRAD-seq has some issues with the representability of the actual studied genome, ddRAD-seq can be very useful for estimating parameters that carry information on how adaptation occurs, such as population structure and gene flow (Andrews, Good, Miller, Luikart, & Hohenlohe, 2016; Lowry et al., 2017; Peterson, Weber, Kay, Fisher, & Hoekstra, 2012). Here, we used these proxies to understand the mechanism that maintains genetic similarity across populations such as gene flow. However, to find the punctual areas of the genome under selection, we recommend using

genetic approaches where the genome representation does not get reduced, such as whole-genome approaches.

4. *P. fuscatus integrates information from multimodal signals according to certain social requirements*. Signals are the building blocks in communication (Hebets & Papaj, 2005); here, we studied how *P. fuscatus* integrate these building blocks in certain social contexts. We showed that *P. fuscatus* workers use chemical signals over visual signals for nestmate recognition (class-level recognition). Visual signals in *P. fuscatus* have been previously linked to individual recognition (Sheehan & Tibbetts, 2011; Tibbetts, 2002, 2004). Our results suggest that despite the lack of large brains in *P. fuscatus* (wasps' number of neurons = ~ 960,000; humans = 100 billion) (Herculano-Houzel, 2009; Menzel & Giurfa, 2001), *P. fuscatus* can perform complex social tasks such as signal integration for nestmate recognition. These types of abilities are present in phylogenetically distant groups such as primates (Bergman & Sheehan, 2013). Future research should study the neurological basis of signal integration in *P. fuscatus*. We recommend directed experiments to test how learning and memory mediate the integration of complex signals and if these mechanisms are shared across phylogenetically distant taxa with similar social needs.

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