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Geographical variation in signals and responses: individual identity signals linked with capacity for individual face learning across *Polistes fuscatus* wasp populations

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Communication requires both signals and receiver responses, yet we know little about how signals and responses covary when recognition capacity differs across a species' geographical range. Previous work has shown that *Polistes fuscatus* wasps from Michigan, U.S.A. are capable of individual face recognition, while *P. fuscatus* from central Pennsylvania, U.S.A. are not. Here, we provide a broader assessment of intraspecific variation in signals and responses across the range of *P. fuscatus*. We quantify the amount of perceivable facial pattern variation in each population as a measure of individual identity signalling. We also measure how accurately wasps from each population learn to discriminate between a standard set of conspecific faces as a measure of individual face recognition. We show that wasps are equally adept at learning to discriminate faces of wasps from their own population and faces of wasps from different populations, confirming that discriminating a standard set of faces provides a comparable measure of individual face learning capacity. We find that there is striking variation in signals and responses across wasp populations and that individual identity signals and receiver responses are linked. Wasps from populations with more variable individual identity signals learn to discriminate between a standard set of conspecific faces more accurately than wasps from populations with less variable individual identity signals. Overall, we find surprisingly high levels of intraspecific geographical variation in individual identity signals and responses. Work in additional taxa will be important to assess whether signal phenotype and receiver responses are commonly linked in geographically variable communication systems.

A fundamental question in evolutionary biology is how traits evolve when selection on one individual depends on the phenotype of other individuals (e.g. mutualism, predator–prey relationships, cooperation, communication) (Herre et al., 1999; Moore et al., 1997). Communication requires both signals and receiver responses (Bradbury & Vehrencamp, 1998; Searcy & Nowicki, 2005). Selection on senders depends on receiver phenotypes and vice versa. Senders must have variable phenotypes that convey information to receivers and receivers must pay attention to these phenotypes and respond appropriately. Either component alone is insufficient (Scott-Phillips et al., 2012). For example, humans with unique facial features that allow individual recognition will benefit if receivers learn and remember them but not if receivers are incapable of recognition. Similarly, the ability to recognize individuals may be beneficial if senders have variable, unique phenotypes, but not if senders are so similar that individual recognition is impossible. Therefore, a change in either signals alone or responses alone could disrupt a communication system. Consequently, a positive correlation between signals and responses might be expected in the evolution of communication systems.

Because communication requires both signals and responses, there has been much interest in how both traits vary across species' geographical ranges. Thus far, most work has focused on geographical variation in signals involved in mate choice, including visual, acoustic and chemical signals in birds, fish, mammals and insects (Foster & Endler, 1999; McElfresh & Millar, 2001; Podos & Warren, 2007; Searfoss et al., 2020). Sexual signals and receiver responses are often linked (Panhuis et al., 2001). For example, birdsong dialects often vary geographically and adults preferentially choose mates that sing the local dialect (Slabbekoorn & Smith,

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2002a). Similarly, túngara frog, *Physalaemus pustulosus*, calls differ across their range and females prefer the calls of local males (Pröhl et al., 2006). However, in some taxa, signals and responses are not linked, as females prefer males with the most elaborate ornaments, even if the extent of male ornament elaboration varies across a species' range (Hill, 1994).

Theory suggests intraspecific variation in sexual signals and receiver responses are often linked across geography because signals and responses are genetically correlated. In taxa with genetic covariance between signal and receiver response, selection on either trait leads to a change in the other trait. Genetic covariance between mating signals and mate preferences has been found in many sexual signalling systems (Bakker & Pomiankowski, 1995). It arises due to assortative mating between senders with ornaments and receivers that prefer the ornaments (Fowler-Finn & Rodríguez, 2016). Choosers with extreme mating preferences mate with courters that have extreme ornaments, so preference and ornaments become genetically linked. Consistent with theory, a recent review found that sexual signals are commonly genetically correlated with mate preferences (Fowler-Finn & Rodríguez, 2016).

While there has been significant progress in understanding intraspecific geographical variation in signals and responses in sexual communication systems, we lack parallel theoretical and empirical work in nonsexual communication systems. On one hand, having both signals and receiver responses is crucial for communication, so the traits may often be associated. On the other hand, there is no assortative mating in nonsexual signals, so genetic correlation between signals and responses may be unlikely. Instead, signals and responses could be linked via other mechanisms, like 'reciprocal plasticity' (Tibbetts & Snell-Rood, 2021). Thus far, we lack data testing whether and how nonsexual signals and receiver responses are linked.

Individual recognition is one type of communication system that varies geographically, but we currently know little about the how signals and responses covary. During individual recognition, receivers learn the unique phenotype of conspecifics, associate the phenotype with individual-specific information and recall the phenotype—information link during subsequent interactions (Tibbetts & Dale, 2007). The variable phenotypes used for individual recognition are called individual identity signals. Some individual identity signals vary geographically. For example, orangutan vocal calls used to individually identify neighbours (Spillmann et al., 2003) vary in their duration and pulse rate across populations (Delgado, 2007). Similarly, parrot contact calls and dolphin signature whistles have different characteristics across populations (Janik et al., 2006; Wright, 1996). Importantly, although the precise characteristics of the individual identity signals vary across populations, all populations have highly variable individual identity signals and are assumed to be capable of individual recognition. Comparatively little is known about whether receiver capacity for individual recognition differs across populations. To our knowledge, *Polistes fuscatus* paper wasps are the only taxon known to show different capacity for individual recognition across populations (Tibbetts et al., 2021).

Polistes fuscatus offer an intriguing system to test how individual identity signals and capacity for individual recognition covary geographically. Previous work has shown that *P. fuscatus* vary in both individual identity signals and capacity for individual recognition. In Michigan and New York, U.S.A., *P. fuscatus* have highly variable facial patterns that signal individual identity (Fig. 1) (Tibbetts, 2002). These wasps are adept at learning and remembering the unique facial patterns of conspecifics. Wasps use individual recognition during social interactions on and off nests (Sheehan & Tibbetts, 2008; Tibbetts, 2002; Tibbetts et al., 2020) and also learn to discriminate between individual conspecific faces during training (Tibbetts, Injaian, et al., 2018). In contrast, *P. fuscatus* from Rothrock, Pennsylvania (Tibbetts et al., 2021) and Louisiana (Tumulty et al., 2023), U.S.A. lack individual recognition. *P. fuscatus* from Pennsylvania do not learn and remember individual conspecifics during social interactions (Tibbetts et al., 2021) and are also not capable of learning to differentiate conspecific faces, even when unique conspecific faces are used for training (Tibbetts et al., 2021). Furthermore, the facial patterns that signal individual identity are less variable in wasps from Pennsylvania than wasps from Michigan (Fig. 1).

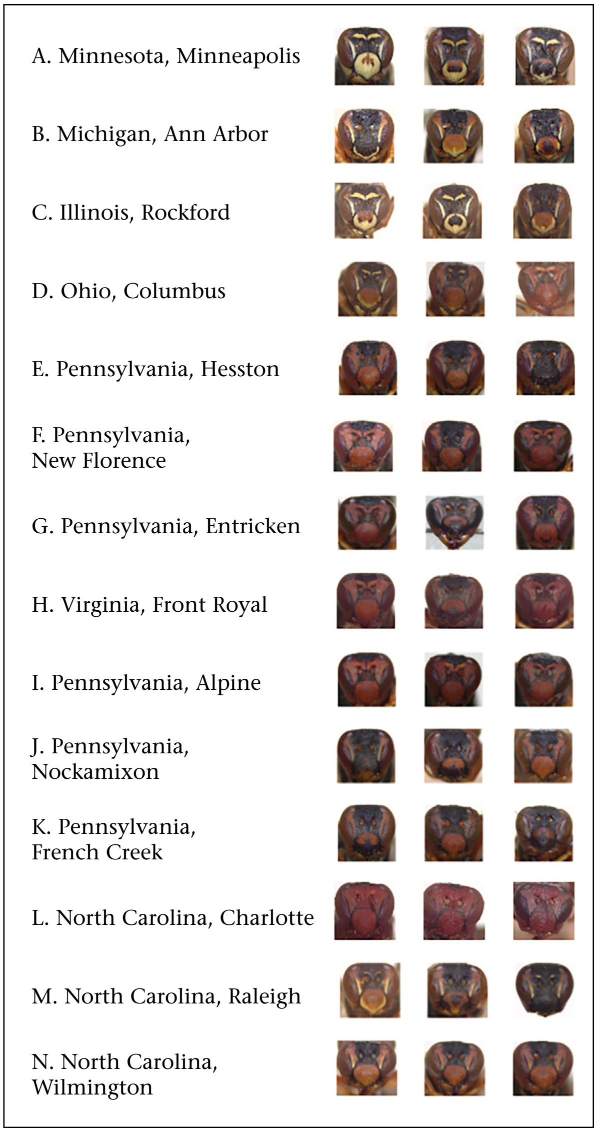

Figure 1. Portraits of *Polistes fuscatus* wasps collected from different locations illustrating the differences in facial pattern variation between populations.

Here, we explore the factors that influence variation in individual identity signals and capacity for individual face recognition at multiple sites across the geographical range of *P. fuscatus*. This study expands our previous work comparing two *P. fuscatus* populations (Tibbetts et al., 2021) to 14 populations. It also provides an in-depth quantification of wasp facial pattern variation that incorporates information about wasp vision. First, we quantify individual face learning capacity of wasps from each population by assessing how well wasps learn to discriminate between a standard set of conspecific faces. We also confirm that wasps are equally adept at learning to discriminate faces of wasps from their own population and faces of wasps from different populations. Measuring learning of different face stimuli is important to ensure that the standard set of wasp face images provides a comparable measure of individual face learning capacity. Next, we assess variation in individual identity signals by quantifying the level of within-population variation in facial patterns. High levels of facial pattern variation facilitate individual discrimination and provide more effective individual identity signals than lower levels of facial pattern variation (Dale et al., 2001; Tibbetts et al., 2017). Finally, we test whether there is a link between individual identity signal variation and individual face learning across nine populations of *P. fuscatus*. We predicted that populations with greater facial pattern variation would be more adept at learning a standard set of face images than populations with less facial pattern variation.

METHODS

Collection

Polistes fuscatus wasps were collected from 14 populations across the eastern United States (Michigan, Minnesota, Illinois, Ohio, Virginia, Pennsylvania and North Carolina) (Fig. 2). After collection, wasps were transported to Ann Arbor, Michigan. Wasps and their nests were housed in individual containers and fed water, sugar and waxworms ad libitum.

Wasps used in the trials were collected as adults from the wild. Wasps collected prior to worker emergence were categorized as nest-founding queens. Wasps collected after worker emergence were dissected to assess caste. Wasps were classified as queens if their ovaries contained eggs that were larger than 1 mm and classified as workers if their ovaries contained eggs that were smaller than 1 mm (Tibbetts et al., 2011). Differences in ovarian development between queens and workers are not subtle, so caste discrimination was straightforward. Only one wasp per nest was used for individual identity signal assessment to ensure that any similarity in facial patterns within a nest did not influence population measures of facial pattern variation.

Individual Identity Signal Assessment

Photography

To measure signal variation, we first photographed all wasp faces using a Leica microscope that had a camera attachment, with the same magnification and lighting for all photographs. To photograph the wasp's faces unobstructed, antennae were removed prior to photography. To minimize potential visual distortion, water droplets were removed from the wasp faces with KimWipes. To control for variation in lighting across photographs, we white-balanced each image using built-in functions in MatLab (‘illumgray’ and ‘chromadapt’; Mathworks, Natick, MA, U.S.A.). Note that, because we used image segmentation and colour remapping later in image processing, white-balancing had no effect on our results.

Facial analysis

To control for variation in face shape, we used a custom script in MatLab (version R2020B, (https://www.mathworks.com/help/images/ref/fitgeotrans.html) to transform all wasp faces to align with a template face chosen because the face picture had ideal orientation (PA-GF193). To do this, each wasp face was marked with a series of landmark points signifying reliably identified facial features (inner eye, bottom of clypeus, top of upper frons). We also applied a mask to each image, which allowed us to remove parts of the face that do not vary between populations (the eyes, lower mandible) (Tibbetts et al., 2021). A projective transformation was then applied to the focal wasp image to warp it, so that its landmarks lined up with the corresponding landmarks on the template. This type of transformation is best used when most of the differences between images are the result of one image being slightly tilted relative to the other images, which was the case for our images. Finally, we applied a low-level Gaussian blur to the masked images to account for the low visual acuity of a wasp. The warped and blurred output images were exported from MatLab to be used for subsequent analysis in R.

Colour adjustments and shine removal

To ensure that our analysis captured variation that would be visually discernible to wasps, we remapped wasp colour space values to each pixel on the wasps' face. To do this, we first used the R

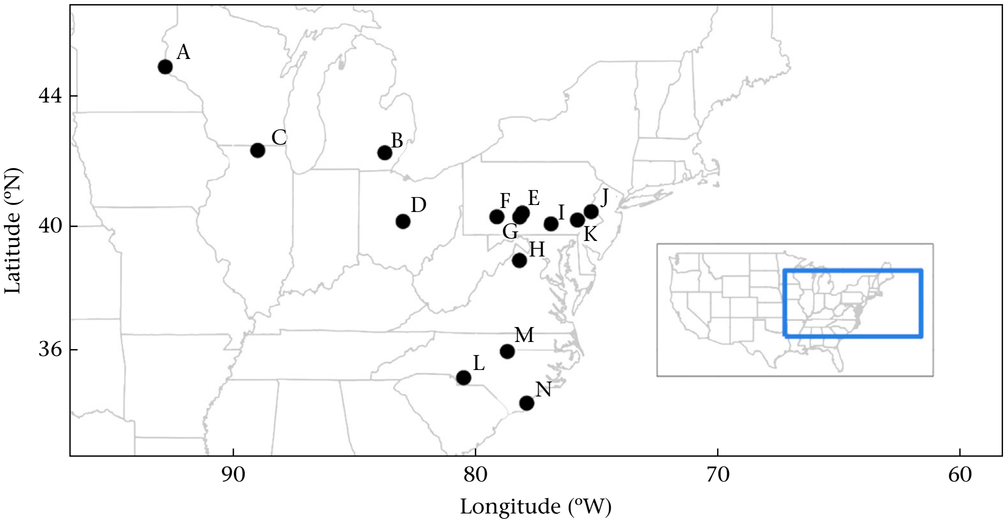

Figure 2. Map of collection locations. A: Minnesota, Minneapolis; B: Michigan, Ann Arbor; C: Illinois, Rockford; D: Ohio, Columbus; E: Pennsylvania, Hesston; F: Pennsylvania, New Florence; G: Pennsylvania, Entricken; H: Virginia, Front Royal; I: Pennsylvania, Alpine; J: Pennsylvania, Nockamixon; K: Pennsylvania, French Creek; L: North Carolina, Charlotte; M: North Carolina, Raleigh; N: North Carolina, Wilmington.

package ‘recolorize’ (https://CRAN.R-project.org/package=recolorize) to perform colour segmentation on the face images, which provided us with a uniform colour palette for each population. Recolorize uses segmentation algorithms to extract distinct colours from an image, which is particularly useful when analysing images of species with a limited colour palette, as in the case of *P. fuscatus* (Weller et al., 2024). We used the ‘hist’ method with five bins to segment the colours, then applied a clustering algorithm to group similar colours together. Specifically, we used the ‘recluster’ function to perform hierarchical clustering on the colour values in the ‘Lab’ colour space, which is designed to be more perceptually uniform than other colour spaces. The clustering algorithm was based on the Euclidean distance between the colours, and we set the cutoff distance to 30 to ensure that the algorithm did not merge colours that were too different from each other. The resulting colour palette was then applied to each pixel in the image using the ‘imposeColors’ function, which assigns each pixel to the closest colour in the palette. This image segmentation step allowed us to assign each pixel to one of a set of limited colours (black, brown, dark brown, red-brown and yellow). For example, wasps from North Carolina populations were primarily red-brown and black, with small patches of yellow (Fig. 1).

We then used an Ocean Optics USB400 spectrophotometer along with a custom fibre optic probe to measure the reflectance spectra for the full set of colours from representative individuals across all populations (black: $N = 8$; brown: $N = 3$; dark brown: $N = 3$; red-brown: $N = 16$; yellow: $N = 5$). The number of measurements for some colours was limited because some colour patches were below the smallest area the probe could measure. We previously collected photographs of wasp faces that revealed that *P. fuscatus* wasps have very low reflectance in the ultraviolet (UV), consistent with other research in *Polistes* coloration using both spectrophotometric and multispectral imaging (Jernigan et al., 2023; Miller, 2023). Therefore, we chose to set a low, flat reflectance for the UV range of the spectra. We calculated the average reflectance for each colour between 401 and 700 nm and set the 300–400 nm reflectance as the value at 401 nm. We then used the average reflectance spectra to calculate colour space coordinates, using the hexagonal model of colour vision (Chittka, 1992). This colour space is perceptually uniform and can be used to measure the distances between colours. We calculated wasp spectral sensitivity curves using the function ‘sensmodel’ in the R package ‘pavo’ and the published photoreceptor maximum sensitivities of *Polistes dominulus* (van der Kooi et al., 2021). Photoreceptor sensitivities for *P. fuscatus* are not known but sensitivities for a congeneric species are expected to be a good approximation, given that sensitivities do not vary widely among closely related taxa (van der Kooi et al., 2021). Each pixel in an image was then changed to the colour space coordinate values that corresponded to its assigned colour.

Eigenfaces (principal component) analysis

To assess variation within and across populations, we ran principal component analysis (PCA) of the warped and recolourized faces. We converted each image to a string of pixel values and performed PCA on these strings. This produces a ‘wasp face space’ where the distance between the coordinates for any pair of faces represents how similar the faces are. To quantify facial variation, we calculated the average pairwise distance between faces within each population for the first 10 PCAs. Populations with tight clusters of faces should have a lower variation metric than populations with a range of facial patterns with faces spread widely across wasp face space. We analysed the following number of *P. fuscatus* face pictures in each population: Ann Arbor, Michigan: $N = 70$; Minneapolis, Minnesota: $N = 26$; Rockford, Illinois: $N = 17$; Columbus, Ohio: $N = 11$; Front Royal, Virgina: $N = 8$; Hesston, Pennsylvania: $N = 20$; New Florence, Pennsylvania: $N = 7$; Entriken, Pennsylvania: $N = 12$; Alpine, Pennsylvania: $N = 8$; French Creek, Pennsylvania: $N = 11$; Nockamixon, Pennsylvania: $N = 9$; Charlotte, North Carolina: $N = 14$; Raleigh, North Carolina: $N = 17$; Willington, North Carolina: $N = 30$.

Individual Face Learning

We tested how accurately wasps learned to discriminate between a neutral face stimulus and a negative face stimulus (Tibbetts et al., 2021). This test provides a more fine-grained measure of individual face learning than experiments involving social interactions. Experiments involving social interactions (e.g. Tibbetts, 2002; Tibbetts et al., 2020) provide good information about whether individual recognition occurs, but not the accuracy of recognition. Previous work has shown that the ability to learn and remember individual faces during training is linked to individual recognition capacity. Within and between species, wasps that are capable of individual recognition learn and remember faces during training, while wasps that are not capable of individual recognition are unable to learn and remember faces (Sheehan & Tibbetts, 2011; Tibbetts, Den Uyl, et al., 2019; Tibbetts, Pandit, et al., 2018). For example, *Polistes metricus* wasps, *P. fuscatus* from Michigan that were socially isolated since emergence from pupation and *P. fuscatus* from central Pennsylvania are not capable of individual recognition in social situations and are unable to learn to discriminate faces during training (Sheehan & Tibbetts, 2011; Tibbetts, Den Uyl, et al., 2019; Tibbetts et al., 2021). In contrast, *P. fuscatus* from Michigan and New York are capable of individual recognition and readily learn to discriminate faces (Sheehan & Tibbetts, 2011; Tibbetts, Pandit, et al., 2018).

We trained wasps via classical conditioning by placing them in a 2.5 × 4 cm wooden box, with a Plexiglas top. Along each wall of the box, we placed identical images of a conspecific face. The chamber and wasp were placed on an electrified pad that delivered either 0.4 V of electric shock or no shock to the wasp as she viewed the conspecific face. The chambers were less than 1 cm high so that wasps could not escape the electrical current. In half the trials, wasps were exposed to an incorrect face (CS+) while receiving a mild electric shock for 2 min. In half the trials, wasps were exposed to a correct face (CS−) while receiving no electric shock. The wasp received a 1 min break in a separate container between each trial. The sequence of one CS+ and one CS− trial was repeated three times per wasp, so wasps experienced three CS+ and three CS− trials in total. After training, the wasp was given a 45 min break in a separate container with water.

After training, we measured each wasp's learning accuracy with a 10-trial test. In each trial, the wasp was able to walk towards the correct (CS−) or incorrect (CS+) image. Between each trial, wasps rested for 1 min in a separate container. Performance was measured as the number of correct choices over 10 trials.

Testing occurred in a wooden chamber that was 9.5 × 3 cm long, with Plexiglas on top. One end of the rectangle displayed the correct stimulus (CS−) while the other end of the rectangle displayed the incorrect stimulus (CS+). At the beginning of each trial, the wasp was placed in the middle of the rectangle between two clear partitions for 3 s, then both partitions were removed simultaneously and the wasp was free to walk through the maze. A wasp was scored as making a choice when its head and thorax moved beyond a small partition placed 2.5 cm from each end of the maze. After a wasp made a choice, it was removed from the maze and given a 1 min break in a dark holding container. The placement of the stimuli (right or left side) was determined randomly and changed between trials. This ensured that wasps did not associate a particular direction with correct choices.

Stimuli

Wasps from all populations were trained and tested using a standard set of Michigan *P. fuscatus* face images. *Polistes fuscatus* faces from Michigan were photographed and printed at life size (~3.5 mm wide) to be used as stimuli. The same set of face stimuli were used across all populations to ensure that face learning performance was directly comparable.

We ran a second experiment to test whether wasps' ability to discriminate faces differs when wasps are trained to discriminate faces from their own population or faces from a different population. For the non-Michigan stimuli, we created a standard set of face pictures from three additional populations (Front Royal, VA, Charlotte, NC, and Rockford, IL; see Fig. 2 for population details) and printed the photographs at life size.

DNA Barcoding

In addition to the morphological identification of *P. fuscatus*, we used ‘DNA barcoding’ to confirm the species identities of wasps included in this project. DNA barcoding is a commonly used technique to verify the molecular taxonomic identity of insect species (Hebert et al., 2003). Specifically, we used the mitochondrial *cytochrome c oxidase subunit I* (COI) gene to confirm species identities.

We randomly selected a few wasps from each population for barcoding (Ann Arbor, MI: $N = 6$; Wilmington, NC: $N = 4$; Raleigh, NC: $N = 3$; Charlotte, NC: $N = 6$; Hesston, PA: $N = 4$; Ohio: $N = 5$; Minnesota: $N = 5$; Illinois: $N = 5$; Virginia: $N = 5$). DNA was extracted from the abdominal tissues from each wasp. We followed the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, U.S.A.) manufacturer's protocols for DNA extraction. DNA was eluted with 100 μl DNAse/RNAse free H_2O, and DNA concentration was quantified using Nanodrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, U.S.A.). After confirming successful extraction, we amplified the 658 bp region of the COI region using the primer pair LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3) (Folmer et al., 1994; Hebert et al., 2003). All reactions had a total reaction volume of 20 μl, using 0.025 U of Platinum Taq DNA polymerase (Invitrogen), with 4 μl of DNA template, 1× reaction buffer, 2.5 mM $MgCl_2$, 0.2 μM dNTPs and 0.5 μM primers (each). Samples were amplified at 95 °C for 1 min, followed by 35 cycles of 95 °C for 45 s, 50 °C for 1 min, 72 °C for 1 min and a final extension step of 72 °C for 7 min. PCR products were verified using gel electrophoresis. After amplification we sequenced our samples using Sanger sequencing techniques.

Resulting sequences were edited and aligned in Geneious Prime 2020.0.4. Species identity of samples was confirmed by comparing each sequence for the percentage of identity with previously sequenced *Polistes* wasps on the Barcode of Life Database (BOLD version 4, http://www.boldsystems.org/) using the Animal Identification tool (Ratnasingham & Hebert, 2007). All sequences had >98% sequence similarity with *P. fuscatus* in BOLD, confirming the morphological species identifications.

Ethical Note

Training involves low-level electrical shock that is aversive to wasps. However, the level of current was kept low to ensure that wasps were able to learn and were not harmed. Wasps behaved normally when they were returned to their containers after training. Wasps were euthanized on dry ice prior to facial pattern analysis and DNA barcoding.

Statistics

Statistical analyses were performed in R version 4.1.2 (‘Bird Hippie’) and SPSS statistical software (version 26). Residual diagnostics were performed to assess model fit.

We tested how geographical variation in facial diversity differs across the U.S. with a general linear model. The dependent variable was the facial variation metric within a population ($N = 14$ populations). The independent variables were population latitude (continuous) and longitude (continuous). In a separate analysis, we tested how individual face learning differs across the U.S. with a linear mixed model. The dependent variable was the number of correct choices (out of 10) for 245 wasps from nine populations. The independent variables were population latitude (continuous), longitude (continuous) and caste (categorical). Population was included as a subject variable to control for any similarity within populations.

We also tested whether individual face learning and facial pattern variation was linked using a mixed linear model. The dependent variable was the number of correct choices (out of 10) for 245 wasps from nine populations. The independent variables were population variation (continuous) and caste (categorical). Population was included as a subject variable to control for any similarity within populations, allowing intercepts to vary across populations.

In a second set of experiments, we tested whether the specific face stimuli used during training influenced individual face learning performance ($N = 86$ wasps). We analysed these data by using a general linear model to test whether individual face learning performance (out of 10) was explained by population of origin of the face stimuli pictures (categorical), the population of origin of the wasp being trained (categorical), the interaction between the population of the face stimuli and the population of the wasps being trained and caste (categorical). The interaction was not significant, so it was removed from the final model, although the interaction results are reported below.

RESULTS

Facial patterns differed across the geographical range of *P. fuscatus* (Fig. 1). Populations primarily differed in the amount of colour pattern variation. Facial pattern variation was linked with longitude ($F_{1,11} = 38.7$, $P < 0.001$, SE = 0.031, partial eta squared = 0.173; Fig. 3a), but not latitude ($F_{1,11} = 2.3$, $P = 0.16$, SE = 0.054, partial eta squared = 0.78; Fig. 3b). Wasps from western populations had more variable facial patterns than wasps from eastern populations (Fig. 3a). The type of facial patterns found in a population also differed somewhat. For example, high levels of bright yellow colour were fairly common in Minnesota, but not in more eastern populations. Many facial pattern types were common across the entire range of *P. fuscatus*. For example, most populations contained some wasps with an entirely black face and some wasps with a face that is mostly black with yellow inner eye stripes (Fig. 1).

Individual face learning differed across populations ($F_{8,235} = 26.6$, SE = 0.46–0.60, $P < 0.001$), but not castes ($F_{1,235} = 6.8$, SE = 0.32, $P = 0.088$). We followed up the overall model with a more specific analysis exploring how geographical location and individual face learning are linked, including population as a subject variable to control for any similarity within populations. Wasps from more western populations learned to discriminate facial patterns more accurately than wasps from more eastern populations ($F_{1,241} = 24.3$, SE = 0.033, $P < 0.001$). Latitude was not associated with individual face learning ($F_{1,241} = 1.0$, SE = 0.058, $P = 0.31$), indicating that there was no difference in face learning between northern and southern

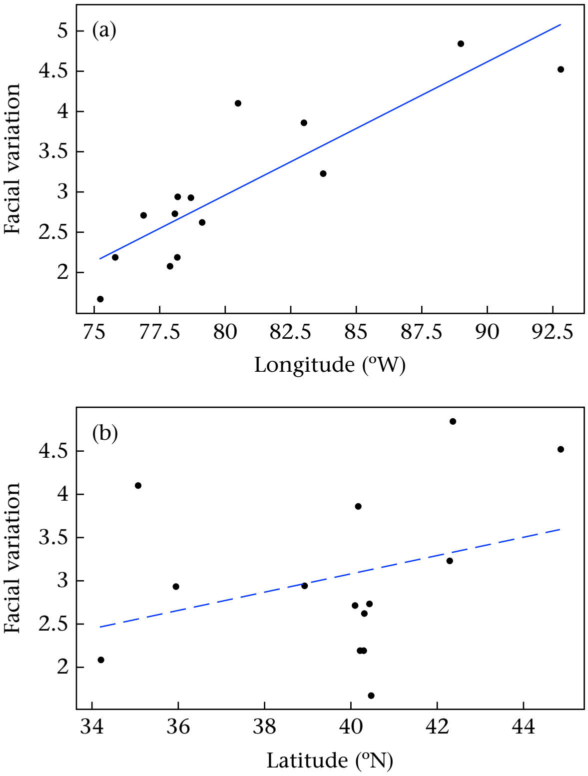

Figure 3. Relation between *P. fuscatus* facial pattern variation and (a) longitude and (b) latitude. Facial variation was measured as the average pairwise distance between faces within each population.

populations. In this model, caste was not linked with individual face learning ($F_{1,242} = 2.7$, SE = 0.30, $P = 0.098$).

Individual recognition requires both highly variable individual identity signals and receivers that can learn to differentiate individuals. As a result, we tested whether sender phenotypes and receiver responses are linked across the range of *P. fuscatus*. Individual face learning was positively linked to colour pattern variation (Fig. 4). Populations with more variable facial patterns were also more adept at learning to discriminate a standard set *P. fuscatus* face images than populations with less variable facial patterns ($F_{1,6.5} = 6.5$, SE = 0.28, $P = 0.04$; Fig. 4). Individual facial learning was also linked to caste, as nest-founding queens learned to discriminate conspecific faces more accurately than workers ($F_{1,219} = 3.8$, SE = 0.30, $P = 0.05$).

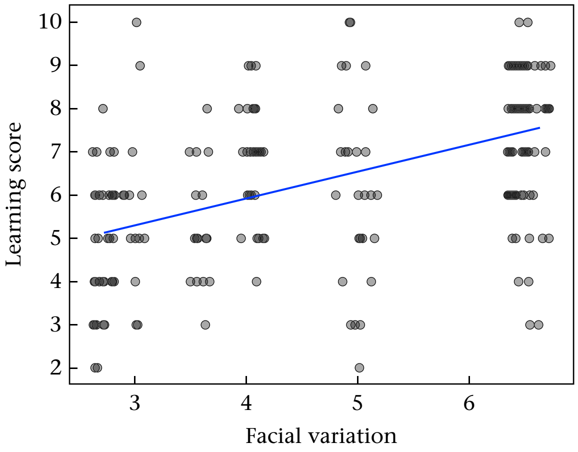

Figure 4. Relation between individual face learning and facial variation in the wasp's population of origin. Facial variation was measured as the average pairwise distance between faces within each population. Dot colour reflects the number of overlapping points, with darker dots reflecting a larger number of overlapping data points than lighter dots.

In a second set of experiments, we confirmed that training wasps from different populations using a standard set of wasp faces did not influence learning performance. We tested whether the specific face stimuli used during training influenced individual face learning by training wasps from multiple populations to discriminate wasp face images from multiple populations. Individual face learning was not influenced by the population of origin of the face stimuli pictures ($F_{3,80} = 0.87$, SE = 0.55–0.59, $P = 0.46$). Furthermore, performance was not influenced by the interaction between the population of the face stimuli and the population of the wasps being trained ($F_{1,79} = 0.10$, $P = 0.75$). The lack of a significant interaction is important because it suggests that wasps from different populations had equal capacity to learn faces from their own population and other populations. Performance was influenced by population of origin of the wasps ($F_{1,80} = 4.1$, SE = 0.38, $P = 0.046$) and caste of the wasps ($F_{1,80} = 6.7$, SE = 0.40, $P = 0.01$). Consistent with other work, wasps from some populations were more adept at individual face learning than wasps from other populations. In addition, nest-founding queens learned more accurately than workers (Tibbetts, Injaian, et al., 2018).

DISCUSSION

Polistes fuscatus wasps showed strong geographical variation in both individual identity signals and individual recognition. We found that the facial patterns that signal individual identity were more variable in western *P. fuscatus* populations than in eastern populations (Fig. 3). High levels of variation facilitated individual discrimination and provided more effective individual identity signals than low levels of variation. The capacity for individual face discrimination also varied across the geographical range of *P. fuscatus*. Wasps from western populations learned to discriminate a standard set of *P. fuscatus* face images with greater accuracy than wasps from eastern populations. In fact, wasps from some eastern populations did not learn to discriminate conspecific faces, as they performed at chance levels during training. Notably, individual identity signals and individual face learning were geographically linked (Fig. 4). Wasps from populations with higher levels of facial pattern variation were more adept at learning to discriminate between a standard set of individual conspecific faces than wasps from populations with lower levels of facial pattern variation. DNA barcoding showed that wasps from all populations were *P. fuscatus*, so differences in signals and receiver responses reflect intraspecific geographical variation.

One interesting aspect of this study was finding that signals and receiver responses were linked across populations. Wasps from populations with more variable facial patterns were more adept at learning to discriminate between individuals than wasps from populations with less variable facial patterns (Fig. 4). The close match between signals and responses is notable given that the developmental basis of these traits is quite different. While geographical variation in signals and receiver responses is widespread, assessing both signals and responses is time-consuming and challenging. As a result, few studies have provided a fine-grained assessment of signals and receiver responses across multiple populations. The studies that have been done focus on sexual signals, where signals and responses are often genetically correlated due to assortative mating (Fowler-Finn & Rodríguez, 2016; Scordato & Safran, 2014). Social signalling systems used in non-mating contexts lack assortative mating, so mechanisms other than genetic correlation must produce the link between signal and receiver response. Additional work in diverse taxa and signalling systems will be important to assess whether there are consistent links between signal phenotypes and receiver responses across taxa and signalling systems or whether mismatches between signals and responses are common.

Plasticity and local genetic adaptation are two mechanisms that could maintain links between signals and responses in nonsexual signalling systems. Plasticity may link signals and responses because plasticity allows individuals to rapidly adjust their phenotype to their partner's phenotype via ‘reciprocal plasticity’ (Tibbetts & Snell-Rood, 2021). For example, animals improve their ability to perceive and learn stimuli when they encounter stimuli more frequently (‘perceptual learning’) (Watanabe et al., 2001). As a result, exposure to stimuli, like highly variable facial patterns, may increase an animal's ability to learn and remember that stimuli (Seitz & Watanabe, 2005; Tibbetts, Den Uyl, et al., 2019; Tibbetts, Pandit, et al., 2018). Receivers can also learn which traits provide reliable information through experience (McLinn & Stephens, 2006), so they can attend to the most relevant sender traits in their population. Local adaptation may also produce links between signals and receiver responses. Populations may experience divergent natural selection on communication systems or cognitive ability because of population differences in traits like predation risk, cooperation rate or habitat characteristics. Divergent selection can lead to local adaptation of signals and responses, although factors like gene flow, drift and the genetic architecture of the traits influence the extent and stability of local adaptation (Kawecki & Ebert, 2004; Tigano & Friesen, 2016).

Geographical variation in coloration is often due to plastic responses to the rearing environment, but our data suggest that plasticity does not play an important role in *P. fuscatus* individual identity signal variation. Environmental conditions often influence coloration, with some taxa showing developmental plasticity in melanization in response to temperature variation (Clusella Trullas et al., 2007). For example, cool temperatures induce cuticle melanization in many insects (de Souza et al., 2016; Kingsolver & Huey, 1998). The variation we observed in *P. fuscatus* is not consistent with prior research on thermal melanization, as the most northern population (Minnesota) have the most yellow and the least black coloration. Furthermore, current research suggests that relatively little of the within-population variation in *P. fuscatus* facial patterns is due to plasticity (Sheehan et al., 2017).

Although there is currently little evidence of plasticity in sender individual identity signals, geographical variation in receiver individual recognition behaviour is likely to be more strongly influenced by plasticity. Previous work has shown that individual recognition in *P. fuscatus* is highly responsive to the environment (Pardo-Sanchez et al., 2022; Tibbetts, Den Uyl, et al., 2019). Newly eclosed wasps develop the capacity for individual face recognition over days of interacting with conspecifics that have variable facial patterns. Wasps that are socially isolated (Tibbetts, Desjardins, et al., 2019) or reared with heterospecifics (Tibbetts, Den Uyl, et al., 2019) are not capable of individual face recognition. Furthermore, *P. fuscatus* from a Pennsylvania population that lacks individual recognition became more adept at individual face learning when reared with *P. fuscatus* from a Michigan population that has individual recognition (Simons et al., 2024). Therefore, the geographical differences in individual recognition are likely due, in part, to plastic responses to differences in rearing environment between populations, including exposure to wasps with variable facial patterns and/or differences in social interaction.

Differences in cooperation across populations may be a key factor the drives the geographical variation in individual recognition and individual identity signals. Individual recognition facilitates social interactions among cooperative nest-founding queens (Tibbetts, 2002; Tibbetts et al., 2020). As a result, populations with higher levels of cooperation among queens may experience greater benefits associated with both individual identity signalling and individual recognition, leading to local adaptation. Cooperative behaviour among nest-founding queens varies across *P. fuscatus* populations (Sheehan et al., 2015). For example, *P. fuscatus* from Pennsylvania are less cooperative (mean foundresses per nest = 1.2) than *P. fuscatus* from Michigan (mean foundresses per nest = 2.0). Tumulty et al. (2023) found evidence that cooperation and individual recognition may be linked in *P. fuscatus*, as they found a geographical cline in cooperation rate, facial pattern variation and selection in genes that may be associated with recognition.

The results of this study differ somewhat from recent work that explored geographical variation in *P. fuscatus* facial patterns and cooperative behaviour (Tumulty et al., 2023). Both Tumulty et al.'s study and the present study examined geographical variation in *P. fuscatus* facial pattern, although other aspects of the two studies differ. The present study assessed geographical variation in receiver behaviour, while Tumulty et al.'s study focused on cooperation rates. Both studies found large differences in facial pattern variation between populations. Some populations had low levels of facial pattern variation, with most wasps having entirely brown or black facial patterns (Fig. 1). Other populations had more facial pattern variation, with large differences in the colour, size and shape of patterns in the inner eye, ‘eyebrow’ and clypeus in different wasps.

Our present study and Tumulty et al.'s study differed in the precise facial variation values, which is unsurprising because our methods differed. We measured colour using a spectrophotometer, while Tumulty et al. assessed colour using camera values. We converted colour values to wasp hexagonal colour space to account for wasp colour vision and applied a Gaussian blur to account for the low visual acuity of a wasp, while Tumulty et al. did not. There were also some differences in the pattern of variation across studies: Tumulty et al. found a north–south gradient in facial pattern variation whereas in the present study, we found an east–west gradient. Furthermore, we found that some Pennsylvania populations had relatively low levels of facial pattern variation, while Tumulty et al. did not. There are both methodological and biological reasons for the different patterns of variation. Methodologically, our study and Tumulty et al.'s study measured wasps from different locations (both different specific populations and different geographical ranges) and there can be surprisingly large differences in facial variation between close populations (Fig. 2). Furthermore, we measured one wasp per nest to ensure that any facial pattern similarity within nests did not influence measures of variation within populations, while Tumulty et al. did not. Biologically, the real pattern of geographical variation in individual identity signals is likely based on a biologically relevant gradient in behaviour, like co-founding rate, rather than latitude and longitude. Latitude and longitude merely provide a straightforward way to quantify and describe population differences.

A secondary result from the present study was that *P. fuscatus* were equally adept at learning to discriminate the faces of conspecifics from their own population and conspecifics from a different population. This result is important because the capacity for individual face learning in each population was assessed using a standard set of consistent stimuli. The use of consistent stimuli ensures that results are comparable across populations with different degrees of facial pattern variation. Some previous work in other taxa has shown that recognition within a population is more effective than recognition between populations. For example, human face recognition improves with experience, so individuals are typically better at differentiating faces from their own population than they are at differentiating faces from a different population (Tanaka et al., 2004). Similarly, many birds respond more strongly to songs with the local dialect than to songs from a distant dialect (Baker et al., 1981; Slabbekoorn & Smith, 2002b). However, we found no differences in individual face learning between faces from different populations. One reason may be that similar types of facial patterns are found throughout the geographical range of *P. fuscatus*. Wasps may also have flexible face learning abilities that allow them to accurately learn any face-like stimuli.

The present study focused on visual signals and responses because previous work has shown *P. fuscatus* only use visual signals for individual recognition. For example experimentally altering visual signals prevents individual recognition (Sheehan & Tibbetts, 2009; Tibbetts, 2002). Wasps can learn about individuals by watching conspecifics, even without the tactile interaction needed to assess chemical profiles (Tibbetts et al., 2020). Previous work on geographical variation in individual identity signals tested capacity for individual recognition across modalities, finding that *P. fuscatus* from Pennsylvania are not capable of individual recognition in any sensory modality. Also consistent with visual recognition, wasps capable of individual recognition are adept at learning conspecific faces, while wasps that lack individual recognition cannot learn conspecific faces (Sheehan & Tibbetts, 2011; Tibbetts, Desjardins, et al., 2019). Wasps do communicate in other sensory modalities (chemical, acoustic) (Singer & Espelie, 1992; Izzo et al., 2010; van Zweden & d'Ettorre, 2010). However, there is no evidence of nonvisual individual recognition in *Polistes*.

Communication research often focuses on one or a few populations and considers the behaviour of the population to represent the entire species. However, the present study adds to a growing body of evidence that geographical variation in communication systems is surprisingly widespread. Therefore, communication is not a stable evolutionary end point. Instead, communication systems may rapidly evolve, leading to extensive variation within and between species. Such geographical variation provides an exciting model for studying the factors that mediate the diversification of complex, interdependent phenotypes.

Author Contributions

Chloe Weise: Conceptualization, Data curation, Formal analysis, Investigation, Visualization, Writing – original draft. **Jarome R. Ali:** Formal analysis, Investigation, Methodology, Software, Visualization, Writing – review & editing. **Christian Cely Ortiz:** Conceptualization, Investigation. **Elizabeth A. Tibbetts:** Conceptualization, Formal analysis, Funding acquisition, Supervision, Writing – review & editing.

Data Availability

Data are available as Supplementary material.

Declaration of Interest

The authors declare no conflicts of interest.

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Supplementary Material

Supplementary material associated with this article is available, in the online version, at https://doi.org/10.1016/j.anbehav.2024.09.003.

References

Baker, M. C., Spitler-Nabors, K. J., & Bradley, D. C. (1981). Early experience determines song dialect responsiveness of female sparrows. *Science, 214*(4522), 819–821.

Bakker, T. C. M., & Pomiankowski, A. (1995). The genetic basis of female mate preferences. *Journal of Evolutionary Biology, 8*(2), 129–171. https://doi.org/10.1046/j.1420-9101.1995.8020129.x

Bradbury, J. W., & Vehrencamp, S. L. (1998). *Principles of animal communication.* Sinauer.

Chittka, L. (1992). The colour hexagon: A chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. *Journal of Comparative Physiology A, 170*, 533–543. https://doi.org/10.1007/BF00199331

Clusella Trullas, S., van Wyk, J. H., & Spotila, J. R. (2007). Thermal melanism in ectotherms. *Journal of Thermal Biology, 32*(5), 235–245. https://doi.org/10.1016/j.jtherbio.2007.01.013

Dale, J., Lank, D. B., & Reeve, H. K. (2001). Signaling individual identity versus quality: A model and case studies with ruffs, queleas, and house finches. *American Naturalist, 158*(1), 75–86.

de Souza, A. R., Turillazzi, S., Lino-Neto, J., & Santini, G. (2016). Colder environments may select for darker paper wasps. *Biological Journal of the Linnean Society, 120*(3), 700–704. https://doi.org/10.1111/bij.12910

Delgado, R. A. (2007). Geographic variation in the long calls of male orangutans (*Pongo* spp.). *Ethology, 113*(5), 487–498. https://doi.org/10.1111/j.1439-0310.2007.01345.x

Folmer, O., Black, M., Hoeh, L., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology, 3*, 294–299.

Foster, S., & Endler, J. (1999). *Geographic variation in behavior.* Oxford University Press.

Fowler-Finn, K. D., & Rodríguez, R. L. (2016). The causes of variation in the presence of genetic covariance between sexual traits and preferences. *Biological Reviews, 91*(2), 498–510.

Hebert, P. D. N., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences, 270*(1512), 313–321. https://doi.org/10.1098/rspb.2002.2218

Herre, E. A., Knowlton, N., Mueller, U. G., & Rehner, S. A. (1999). The evolution of mutualisms: Exploring the paths between conflict and cooperation. *Trends in Ecology & Evolution, 14*(2), 49–53. https://doi.org/10.1016/s0169-5347(98)01529-8

Hill, G. E. (1994). Geographic variation in male ornamentation and female mate preference in the house finch: A comparative test of models of sexual selection. *Behavioral Ecology, 5*(1), 64–73. https://doi.org/10.1093/beheco/5.1.64

Izzo, A., Wells, M., Huang, Z., & Tibbetts, E. (2010). Cuticular hydrocarbons correlate with fertility, not dominance, in a paper wasp, *Polistes dominulus*. *Behavioral Ecology and Sociobiology, 64*(5), 857–864. https://doi.org/10.1007/s00265-010-0902-7

Janik, V. M., Sayigh, L. S., & Wells, R. S. (2006). Signature whistle shape conveys identity information to bottlenose dolphins. *Proceedings of the National Academy of Sciences of the United States of America, 103*(21), 8293–8297.

Jernigan, C. M., Stafstrom, J. A., Zaba, N. C., Vogt, C. C., & Sheehan, M. J. (2023). Color is necessary for face discrimination in the Northern paper wasp, *Polistes fuscatus*. *Animal Cognition, 26*(2), 589–598. https://doi.org/10.1007/s10071-022-01691-9

Kawecki, T. J., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters, 7*(12), 1225–1241. https://doi.org/10.1111/j.1461-0248.2004.00684.x

Kingsolver, J. G., & Huey, R. B. (1998). Evolutionary analyses of morphological and physiological plasticity in thermally variable environments. *American Zoologist, 38*(3), 545–560. https://doi.org/10.1093/icb/38.3.545

McElfresh, J. S., & Millar, J. G. (2001). Geographic variation in the pheromone system of the saturniid moth *Hemileuca eglanterina*. *Ecology, 82*(12), 3505–3518. https://doi.org/10.1890/0012-9658(2001)082[3505:GVITPS]2.0.CO;2

McLinn, C. M., & Stephens, D. W. (2006). What makes information valuable: Signal reliability and environmental uncertainty. *Animal Behaviour, 71*(5), 1119–1129. https://doi.org/10.1016/j.anbehav.2005.09.006

Miller, S. E. (2023). Facial color diversity of *Polistes* paper wasps. *Insectes Sociaux, 70*, 181–190. https://doi.org/10.1007/s00040-023-00904-9

Moore, A. J., Brodie, E. D., & Wolf, J. B. (1997). Interacting phenotypes and the evolutionary process. 1. Direct and indirect genetic effects of social interactions. *Evolution, 51*(5), 1352–1362. https://doi.org/10.1111/j.1558-5646.1997.tb01458.x

Panhuis, T. M., Butlin, R., Zuk, M., & Tregenza, T. (2001). Sexual selection and speciation. *Trends in Ecology & Evolution, 16*(7), 364–371. https://doi.org/10.1016/S0169-5347(01)02160-7

Pardo-Sanchez, J., Kou, N., & Tibbetts, E. A. (2022). Type and amount of social experience influences individual face learning in paper wasps. *Behavioral Ecology and Sociobiology, 76*(11), Article 148. https://doi.org/10.1007/s00265-022-03257-8

Podos, J., & Warren, P. S. (2007). The evolution of geographic variation in birdsong. *Advances in the Study of Behavior, 37*, 403–458. https://doi.org/10.1016/S0065-3454(07)37009-5

Pröhl, H., Koshy, R. A., Mueller, U., Rand, A. S., & Ryan, M. J. (2006). Geographic variation of genetic and behavioral traits in northern and southern túngara frogs. *Evolution, 60*(8), 1669–1679. https://doi.org/10.1111/j.0014-3820.2006.tb00511.x

Ratnasingham, S., & Hebert, P. D. N. (2007). BOLD: The Barcode of life data system. *Molecular Ecology Notes, 7*(3), 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x. www.barcodinglife.org

Scordato, E. S. C., & Safran, R. J. (2014). Geographic variation in sexual selection and implications for speciation in the barn swallow. *Avian Research, 5*, Article 8. https://doi.org/10.1186/s40657-014-0008-4

Scott-Phillips, T. C., Blythe, R. A., Gardner, A., & West, S. A. (2012). How do communication systems emerge? *Proceedings of the Royal Society B: Biological Sciences, 279*(1735), 1943–1949. https://doi.org/10.1098/rspb.2011.2181

Searcy, W. A., & Nowicki, S. (2005). *The evolution of animal communication*. Princeton University Press.

Searfoss, A. M., Liu, W., & Creanza, N. (2020). Geographically well-distributed citizen science data reveals range-wide variation in the chipping sparrow's simple song. *Animal Behaviour, 161*, 63–76. https://doi.org/10.1016/j.anbehav.2019.12.012

Seitz, A., & Watanabe, T. (2005). A unified model for perceptual learning. *Trends in Cognitive Sciences, 9*(7), 329–334.

Sheehan, M. J., Botero, C. A., Hendry, T. A., Sedio, B. E., Jandt, J. M., Weiner, S., Toth, A. L., & Tibbetts, E. A. (2015). Different axes of environmental variation explain the presence vs. extent of cooperative nest founding associations in *Polistes* paper wasps. *Ecology Letters, 18*(10), 1057–1067. https://doi.org/10.1111/ele.12488

Sheehan, M. J., Choo, J., & Tibbetts, E. A. (2017). Heritable variation in colour patterns mediating individual recognition. *Royal Society Open Science, 4*(2), Article 161008. https://doi.org/10.1098/rsos.161008

Sheehan, M. J., & Tibbetts, E. A. (2008). Robust long-term social memories in a paper wasp. *Current Biology, 18*(18), R851–R852.

Sheehan, M. J., & Tibbetts, E. A. (2009). Evolution of identity signals: Frequency-dependent benefits of distinctive phenotypes used for individual recognition. *Evolution: International Journal of Organic Evolution, 63*(12), 3106–3113.

Sheehan, M. J., & Tibbetts, E. A. (2011). Specialized face learning is associated with individual recognition in paper wasps. *Science, 334*(6060). https://doi.org/10.1126/science.1211334, 1272–1272.

Simons, M., Green, D. A., & Tibbetts, E. A. (2024). Geographic variation in individual face learning based on plasticity rather than local genetic adaptation in *Polistes* wasps. *Behavioral Ecology, 35*(1), Article arad100. https://doi.org/10.1093/beheco/arad100

Singer, T. L., & Espelie, K. E. (1992). Social wasps use nest paper hydrocarbons for nestmate recognition. *Animal Behaviour, 44*(1), 63–68. https://doi.org/10.1016/s0003-3472(05)80755-9

Slabbekoorn, H., & Smith, T. B. (2002a). Bird song, ecology and speciation. *Philosophical Transactions of the Royal Society B: Biological Sciences, 357*(1420), 493–503.

Slabbekoorn, H., & Smith, T. B. (2002b). Habitat-dependent song divergence in the little greenbul: An analysis of environmental selection pressures on acoustic signals. *Evolution, 56*(9), 1849–1858.

Spillmann, B., Dunkel, L. P., Van Noordwijk, M. A., Amda, R. N. A., Lameiraà, A. R., Wich, S. A., & van Schaik, C. P. (2003). Acoustic properties of long calls given by flanged male orangutans (*Pongo pygmaeus wurmbii*) reflect both individual identity and context. *Ethology, 116*(5), 385–395. https://doi.org/10.1111/j.1439-0310.2010.01744.x

Tanaka, J. W., Kiefer, M., & Bukach, C. M. (2004). A holistic account of the own-race effect in face recognition: Evidence from a cross-cultural study. *Cognition, 93*(1), B1–B9.

Tibbetts, E. A. (2002). Visual signals of individual identity in the wasp *Polistes fuscatus*. *Proceedings of the Royal Society of London Series B: Biological Sciences, 269*(1499), 1423–1428.

Tibbetts, E. A., & Dale, J. (2007). Individual recognition: It is good to be different. *Trends in Ecology & Evolution, 22*(10), 529–537.

Tibbetts, E. A., Den Uyl, J., Dwortz, M., & McLean, C. (2019). The development and evolution of specialized face learning in paper wasps. *Animal Behaviour, 147*, 1–7. https://doi.org/10.1016/j.anbehav.2018.10.016

Tibbetts, E. A., Desjardins, E., Kou, N., & Wellman, L. (2019). Social isolation prevents the development of individual face recognition in paper wasps. *Animal Behaviour, 152*, 71–77.

Tibbetts, E. A., Injaian, A., Sheehan, M. J., & Desjardins, N. (2018). Intraspecific variation in learning: Worker wasps are less able to learn and remember individual conspecific faces than queen wasps. *American Naturalist, 191*(5), 595–603. https://doi.org/10.1086/696848

Tibbetts, E. A., Levy, S., & Donajkowski, K. (2011). Reproductive plasticity in *Polistes* paper wasp workers and the evolutionary origins of sociality. *Journal of Insect Physiology, 57*(7), 995–999. https://doi.org/10.1016/j.jinsphys.2011.04.016

Tibbetts, E. A., Mullen, S. P., & Dale, J. (2017). Signal function drives phenotypic and genetic diversity: The effects of signalling individual identity, quality or behavioural strategy. *Philosophical Transactions of the Royal Society B: Biological Sciences, 372*(1724), Article 20160347. https://doi.org/10.1098/rstb.2016.0347

Tibbetts, E. A., Ortiz, C. C., Auteri, G. G., Simons, M., Fearon, M. L., & Knowles, L. L. (2021). Individual recognition and individual identity signals in *Polistes fuscatus* wasps vary geographically. *Animal Behaviour, 176*, 87–98. https://doi.org/10.1016/j.anbehav.2021.03.018

Tibbetts, E. A., Pandit, S., & Nondorf, D. (2018). Developmental plasticity and the origin of novel communication systems: Individual recognition in *Polistes* wasps. *Evolution, 72*(12), 2728–2735. https://doi.org/10.1111/evo.13613

Tibbetts, E. A., & Snell-Rood, E. C. (2021). Reciprocal plasticity and the diversification of communication systems. *Animal Behaviour, 179*, 297–306. https://doi.org/10.1016/j.anbehav.2021.07.017

Tibbetts, E. A., Wong, E., & Bonello, S. (2020). Wasps use social eavesdropping to learn about individual rivals. *Current Biology, 30*(15), 3007–3010.

Tigano, A., & Friesen, V. L. (2016). Genomics of local adaptation with gene flow. *Molecular Ecology, 25*(10), 2144–2164. https://doi.org/10.1111/mec.13606

Tumulty, J. P., Miller, S. E., Van Belleghem, S. M., Weller, H. I., Jernigan, C. M., Vincent, S., Staudenraus, R. J., Legan, A. W., Polnaszek, T. J., Uy, F. M. K., Walton, A., & Sheehan, M. J. (2023). Evidence for a selective link between cooperation and individual recognition. *Current Biology, 33*(24), 5478–5487. https://doi.org/10.1016/j.cub.2023.11.032

van der Kooi, C. J., Stavenga, D. G., Arikawa, K., Belušič, G., & Kelber, A. (2021). Evolution of insect color vision: From spectral sensitivity to visual ecology. *Annual Review of Entomology, 66*(1), 435–461. https://doi.org/10.1146/annurev-ento-061720-071644

van Zweden, J. S., & d'Ettorre, P. (2010). Nestmate recognition in social insects and the role of hydrocarbons. In G. J. Blomquist, & A. G. Bagneres (Eds.), *Insect hydrocarbons: Biology, biochemistry, and chemical ecology* (pp. 222–243). Cambridge University Press.

Watanabe, T., Nanez, J. E., & Sasaki, Y. (2001). Perceptual learning without perception. *Nature, 413*(6858), 844–848.

Weller, H. I., Hiller, A. E., Lord, N. P., & Van Belleghem, S. M. (2024). recolorize: An R package for flexible colour segmentation of biological images. *Ecology Letters, 27*(2), Article e14378. https://doi.org/10.1111/ele.14378

Wright, T. F. (1996). Regional dialects in the contact call of a parrot. *Proceedings of the Royal Society B: Biological Sciences, 263*(1372), 867–872. https://doi.org/10.1098/rspb.1996.0128