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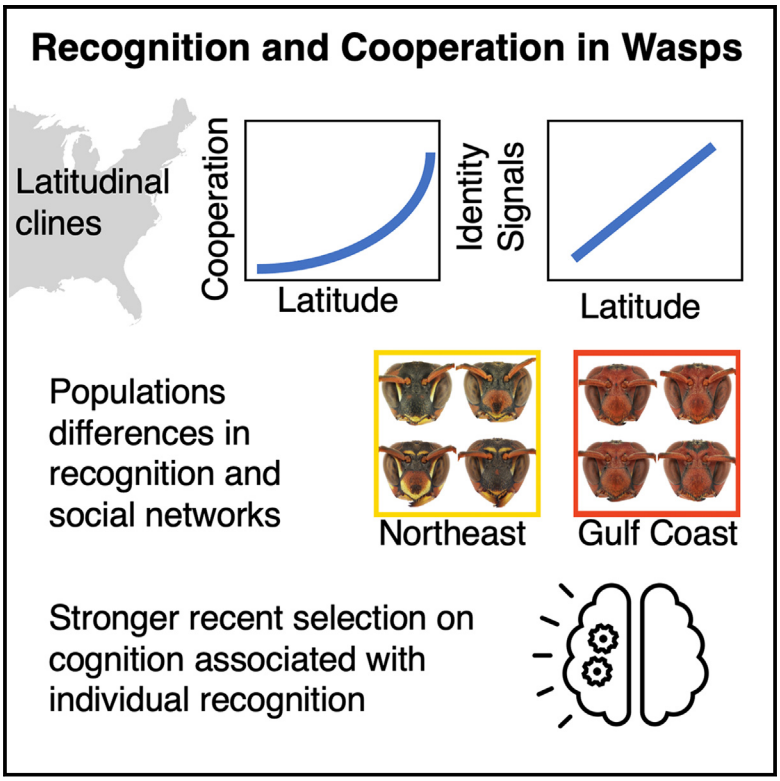
Current Biology

# Evidence for a selective link between cooperation and individual recognition

## Graphical abstract

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## In brief

Using three distinct approaches—a geographic cline of signal diversity and cooperation, common garden behavioral experiments, and genomic analyses of selection—Tumulty et al. show that cooperation favors the evolution of individual recognition in a cooperatively breeding paper wasp.

## Highlights

- Cooperation and signal diversity covary across the geographic range of a paper wasp
- The ability to recognize individuals is related to cooperation and signal diversity
- Individual recognition stabilizes social groups
- Selection on social cognition genes is stronger in more cooperative populations

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# Evidence for a selective link between cooperation and individual recognition

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## SUMMARY

The ability to recognize others is a frequent assumption of models of the evolution of cooperation. At the same time, cooperative behavior has been proposed as a selective agent favoring the evolution of individual recognition abilities. Although theory predicts that recognition and cooperation may co-evolve, data linking recognition abilities and cooperative behavior with evidence of selection are elusive. Here, we provide evidence of a selective link between individual recognition and cooperation in the paper wasp *Polistes fuscatus* through a combination of clinal, common garden, and population genomics analyses. We identified latitudinal clines in both rates of cooperative nesting and color pattern diversity, consistent with a selective link between recognition and cooperation. In behavioral experiments, we replicated previous results demonstrating individual recognition in cooperative and phenotypically diverse *P. fuscatus* from New York. In contrast, wasps from a less cooperative and phenotypically uniform Louisiana population showed no evidence of individual recognition. In a common garden experiment, groups of wasps from northern populations formed more stable and individually biased associations, indicating that recognition facilitates group stability. The strength of recent positive selection on cognition-associated loci likely to mediate individual recognition is substantially greater in northern compared with southern *P. fuscatus* populations. Collectively, these data suggest that individual recognition and cooperative nesting behavior have co-evolved in *P. fuscatus* because recognition helps stabilize social groups. This work provides evidence of a specific cognitive phenotype under selection because of social interactions, supporting the idea that social behavior can be a key driver of cognitive evolution.

## INTRODUCTION

The relationship between cognitive abilities and social structure is of long-standing interest to biologists. The social intelligence hypothesis posits that selection pressures associated with social relationships in complex societies are an evolutionary driver of cognitive complexity.[1–3] Support for this hypothesis comes from comparative studies showing that cognitive performance[4–6] and neuroanatomical proxies for cognition[1,7–9] covary with proxies for social complexity, such as group size or mating system. Recently, general cognitive performance has been linked to group size and fitness in Australian magpies.[10] However, the evidence for the social intelligence hypothesis has come into question because predicted patterns do not hold for some clades and the use of different proxies for cognition and social complexity yields conflicting results.[11–16] More importantly, because of the reliance on such proxies, it has been difficult to identify specific cognitive traits that are under selection to facilitate social interactions.

Models of the evolution of cooperation frequently invoke animal recognition abilities as key mechanisms facilitating the evolution of cooperative behaviors,[17–20] especially in social environments in which animals are likely to encounter a range of potential social partners.[21] Whereas kin recognition facilitates cooperation between relatives,[22] individual recognition has been identified as a building block of social cognition because it allows for cooperation between unrelated individuals.[23] Although the specificity of individual recognition varies across species and contexts,[24] an essential component is discriminating among individuals using individually distinctive phenotypic characteristics.[25–27] Individual recognition allows animals to learn and remember the individuals

they have interacted with previously. Theory indicates that individual recognition enables cooperation because it allows for the identification of group members and reciprocity between individuals.[17,18,28,29] Indirect evidence of the fitness benefits of recognizing familiar individuals comes from studies showing that territorial animals have higher reproductive success when they have familiar neighbors,[30–32] presumably due to benefits associated with the "dear enemy" effect.[33,34] Overall, a major limitation to our understanding of the evolution of social cognition is evidence of a selective advantage of individual recognition in cooperative groups.

Here, we test the hypothesis that cooperative nesting selects for individual recognition in the northern paper wasp (*Polistes fuscatus*). This species provides an excellent study system for understanding the relationship between individual recognition and cooperation because both behaviors have been reported to vary across populations of this species.[35,36] Female *P. fuscatus* found nests in the spring, either as solitary foundresses or cooperatively with other foundresses. When females found nests cooperatively, they establish an aggression-based dominance hierarchy with dominant foundresses laying the majority of the eggs.[37–39] Conflict among co-foundresses manifests in aggression between individuals and egg-eating.[40] Experimental tests of recognition abilities in *P. fuscatus* demonstrate that they not only remember individuals that they have previously interacted with[41,42] but also distinguish among familiar individuals based on their relative dominance status.[43] Individual recognition has been hypothesized to function as a behavioral mechanism that facilitates the maintenance of stable dominance hierarchies and minimizes conflict among individuals across taxa,[44,45] and has been proposed to be important in *P. fuscatus* specifically.[46] The evolution of individual recognition in *P. fuscatus* is associated with increased phenotypic diversity due to the evolution of individually distinctive facial color patterns, which function as identity signals and facilitate recognition[47,48] as well as perceptual and cognitive mechanisms related to recognition.[43,49] However, a selective link between cooperation and individual recognition has yet to be demonstrated. Within-species variation in individual recognition and patterns of cooperation in *P. fucsatus*[35,36] provides a powerful system to test for an evolutionary relationship between the two traits. In this paper, we test the hypothesis that cooperation selects for individual recognition using a combination of approaches: (1) an analysis of geographic clines in identity signaling and cooperation, (2) common garden behavioral assays of individual recognition and grouping behavior between populations with and without identity signals, and (3) population genomic analyses of the strength of selection on cognition-associated loci. These three distinct lines of evidence are all consistent with an evolutionary scenario where selection for stable cooperative associations among paper wasp co-foundresses has selected for individual recognition, an evolutionarily novel cognitive ability in northern *P. fuscatus* populations.

## RESULTS

### Geographic variation in cooperation rates and identity signal diversity in *P. fuscatus*

Variable face color patterns enable individual recognition in northern populations of *P. fuscatus* by serving as individual identity signals.[41,42,47,48] If cooperative nesting has been a selective agent favoring the evolution of individual recognition in *P. fuscatus*, then identity signals should co-vary with rates of cooperative nesting across the species range, with regions with higher rates of cooperative nesting also showing greater color pattern diversity. We collected *P. fuscatus* from across much of its geographic range in the eastern US and discovered striking differences in the amount of within-population color pattern variation (Figure 1A). Using whole genome resequencing, we confirmed that wasps collected from across the range form a monophyletic clade and thus belong to one species, with wasps from southern populations interspersed with more northern populations (Figures 1B, S1A, and S1B). Wasps from southern populations are weakly diverged from northern populations (e.g., New York and Louisiana, $F_{ST}$ = 0.07), matching previous findings of long-distance gene flow in *P. fuscatus*.[50]

We observed nesting behavior in southern wasp populations of *P. fuscatus* and added these data to previously published datasets of nesting behavior in this species.[35,51] Analyzing these data across the range we found a positive relationship between the number of foundresses per nest and latitude ($z = 6.81$, $p < 0.001$, $n = 2{,}021$ nests; Figure 1C), consistent with the findings of earlier studies.[35] At the southern end of the range, approximately 75% of nests are single foundress nests, and the majority of individual wasps nest solitarily (e.g., 56% solitary in Louisiana; Figure 1D). At the northern end of the range, a majority of nests are still single foundress nests, but multi-foundress nests are more common than in southern populations and the majority of wasps in northern populations are part of cooperative groups (e.g., 60% cooperative in New York; Figure 1D). Additionally, the occasional cooperative nests that were observed in the southern portion of the range never had more than three foundresses in our sample of 38 nests observed below 35° latitude. At northern latitudes (above 40° latitude), large nesting associations of 4 or 5 foundresses occur, and groups of 6 or 7 foundresses were occasionally observed in our large sample of nests (Figure 1C).

To measure color pattern diversity, we developed a novel methodology to measure the distance between pairs of face patterns in multi-dimensional "face space," which we briefly outline here (see STAR Methods for details). We photographed antennaless faces and standardized these photographs for slight differences in lighting using the MICA toolbox[52] in ImageJ (Figure 2A). We placed landmarks on the images in homologous locations and then used the patternize R package[53] to align all the images using these landmarks as well as mask out regions of noninterest for color patterns (Figure 2A). We then used a guided color mapping process implemented through the recolorize R package[54] in which we first obtained a color palette of three colors (black, red, and yellow) by binning pixel values based on similarity from a subset of images that had all three colors. Pixels in all images were then classified as black, red, or yellow based on similarity to these three colors in the palette (Figure 2A). The resulting "zone maps" of color[55] were then converted to stacks of three binary rasters, one for each color, and subjected to a principal-component analysis (PCA) transformation using patternize, allowing us to characterize color pattern variation in multi-dimensional space (Figure 2A). Although this method does not incorporate assumptions about how these patterns might appear to conspecific viewers, it does allow for an objective comparison of color

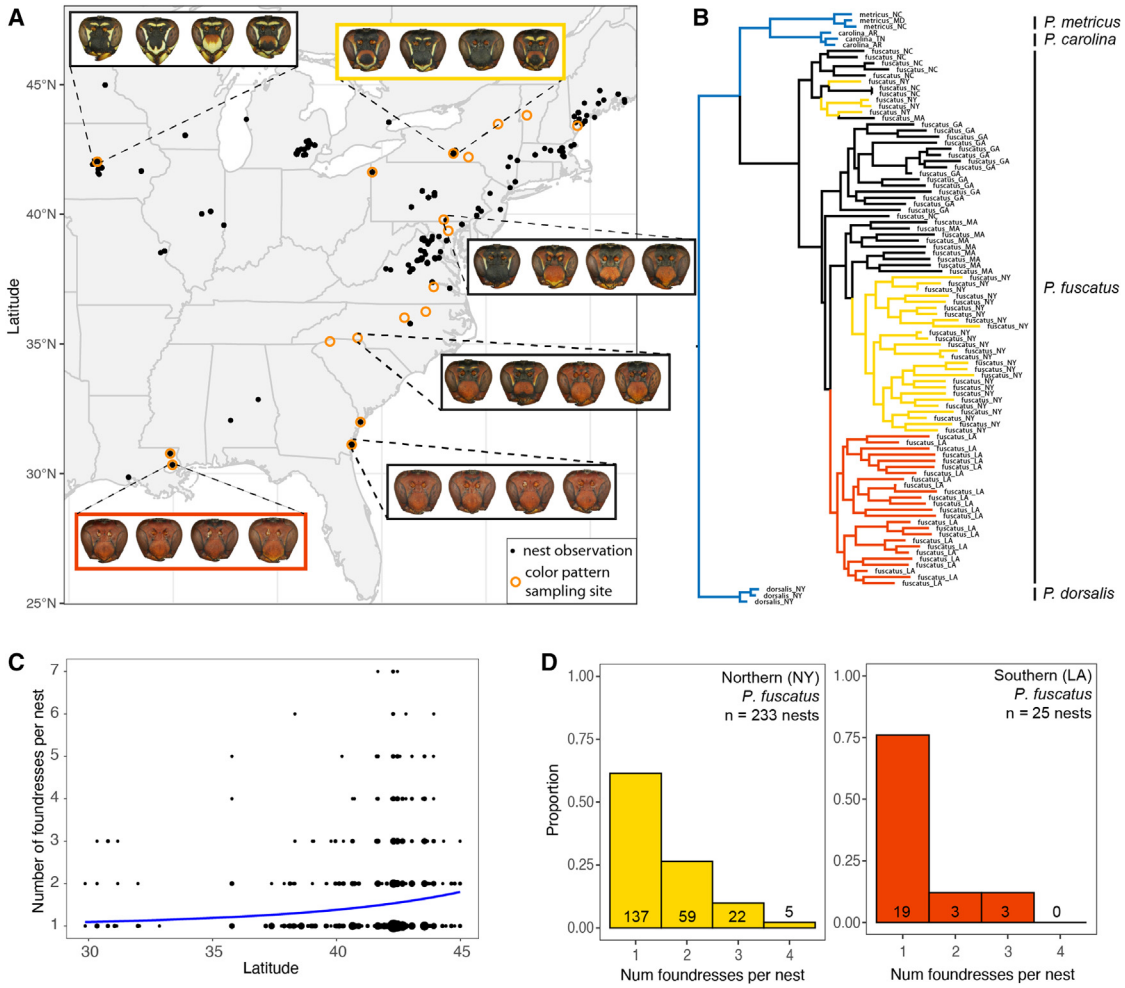



**Figure 1. Intraspecific variation in cooperative nesting**

(A) Map of sampling locations for color pattern diversity and cooperation rates of *P. fuscatus* wasps. Black points represent nest observations taken from the WASPnest dataset[35,51] as well as new observations reported in this paper. Orange open circles mark sites where we collected and photographed wasps to measure color pattern variation. Also shown are photographs of faces of representative individuals from several sites to demonstrate the color pattern variation across the range. Wasps from New York (yellow) and Louisiana (red) are highlighted as representatives of populations that are the focus of the rest of the paper.

(B) A phylogeny generated from SNP data from whole-genome sequencing of *Polistes fuscatus* from across the geographic range (sample information provided in Table S2) confirms that these populations cluster together as a monophyletic clade, indicating that they belong to the same species. Three closely related species (*P. metricus*, *P. carolina*, and *P. dorsalis*) are included as outgroups. Species name and US state of origin are given for each DNA sample. Branches are colored to highlight samples from New York (yellow) and Louisiana (red); samples from North Carolina, Massachusetts, and Georgia are black; and outgroups are colored in blue. See also Figures S1A and S1B.

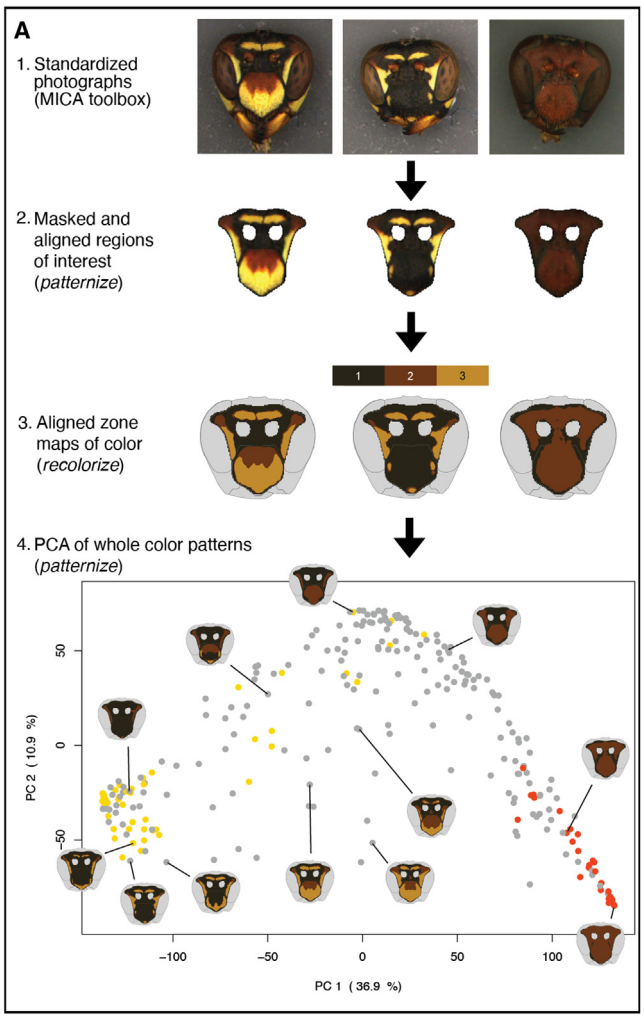
(C) The relationship between the number of foundresses per nest and latitude fit with a zero-truncated Poisson regression line. The sizes of points are scaled according to the number of observations.

(D) Histograms showing the distribution of the number of foundresses per nest in New York and Louisiana populations, showing greater cooperation in New York. Sample sizes of raw numbers of nests observed are shown for each column.

patterns between individuals and sites. We measured face diversity for 18 sites, from which we had photographs of at least 5 individuals (mean = 15, range = 5–35 individuals per site; Figure 1A). To measure face diversity, we computed the pairwise Euclidean distance between faces in PCA space for each site and took the mean of these distances per site. There was a strong positive relationship between latitude and face diversity in a site ($R^2$ = 0.74, $F_{1,\ 16} = 45.9$, $p < 0.001$; Figure 2B). The relative lack of facial diversity was especially pronounced in the southernmost populations from Louisiana and coastal Georgia, which occur below 32° latitude (Figure 2B). Compared with these southernmost populations, face diversity was about 1.6 times higher at around 35° latitude in South and North Carolina, with diversity increasing further in more northern populations (Figure 2B). Results from these two clinal datasets are consistent with the hypothesis that cooperation selects for individual recognition by favoring individuals who signal their identity.

## Recognition abilities are associated with differences in social organization between populations

If the need for cooperation in northern climates has selected for individual recognition, we would expect the wasps from



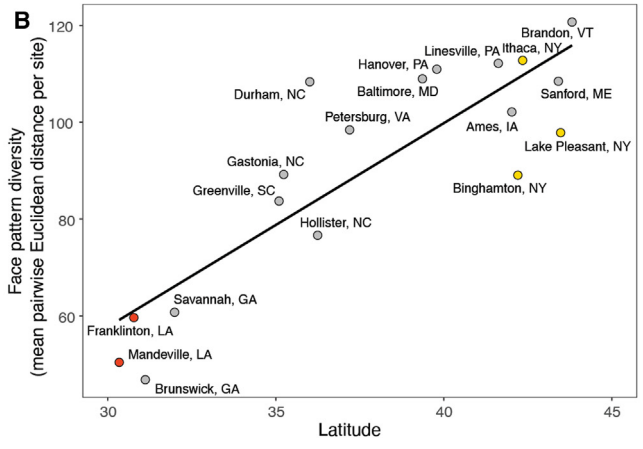




**Figure 2. A novel methodology shows that color pattern diversity correlates with latitude**

(A) (A1) We took digital photographs of wasp faces, with antennae removed to allow a clear view of the color patterns. Photographs were standardized for potential differences in lighting using the MICA toolbox[52] in ImageJ. (A2) We placed landmarks on the images in homologous locations and then used patternize to align the images and mask out regions of noninterest for color patterns. (A3) Pixels in each image were classified as black, red, or yellow, based on similarity to a color palette derived from the images using recolorize. (A4) Binary rasters for each color in the zone map were subjected to a PCA transformation to represent color patterns in multi-dimensional space. The first two principal components (PCs) of 23 statistically significant PCs are shown, as well as some representative faces to visualize how patterns are separated in PC space. A summary of the importance of each significant PC is provided in Table S3.

(B) The relationship between face diversity and latitude across the range of sampling sites fit with a linear regression line. Face diversity was measured as the mean Euclidean pairwise distance between all faces within a population from the PCA scores (shown in A4). Points representing sites in Louisiana are colored red, and those representing sites in New York are colored yellow. See also Figure S4.

cooperative and phenotypically diverse northern populations to show evidence of individual recognition but not wasps from southern populations, which cooperate at lower rates and have lower levels of face diversity. Indeed, previous studies of northern *P. fuscatus* in New York and Michigan have established that foundresses in these populations recognize other individual foundresses,[41,42] and wasps from a population in the central mountainous regions of Pennsylvania with lower levels of pattern diversity did not show evidence of individual recognition.[36] Further, the ability to recognize and discriminate among potential social partners is predicted to shape social networks and influence how animals interact with each other.[56,57] In particular, individual recognition in *P. fuscatus* is hypothesized to be an important behavioral mechanism that enables stable social groups by reducing conflict among co-foundresses with established relationships.[45,46] We tested for individual recognition among wasps from a northern population in New York that has variable faces (Figure 3A) and among wasps from a southern population in Louisiana that has relatively invariant red faces (Figure 3A). These populations represent opposite ends of the latitudinal cline (Figure 1A). We used lab-overwintered individuals, to allow us to compare the behavior of these wasps at the same time, in the same experiment. We also compared the social organization and cooperative nesting behavior of these wasps in freely interacting groups of four wasps in laboratory common garden experiments.

Our individual recognition experiment compared aggression between encounters of familiar and unfamiliar wasps in a neutral arena, following previous studies.[36,42,48,58] Wasps interacted with a new individual (day 0) and again with the same individual 2 days later (day 2, "familiar"). They also interacted with new individuals on days 1 and 3 ("unfamiliar"). This experiment thus asked whether there is a reduction in aggression that is specific to an individual who has been encountered previously (familiar) and controlled for any changes in aggression across days that might not be due to familiarity. We computed an aggression intensity index by weighting observed behaviors by their intensity, following Sheehan and Tibbetts and Dreier et al.[42,48,58] Controlling for experiment day, northern wasps were significantly less aggressive when encountering familiar individuals compared with unfamiliar individuals ($\chi^2 = 10.20$, $p = 0.001$; Figures 3B and S2A). In contrast, southern wasps showed no difference in aggression toward familiar versus unfamiliar individuals ($\chi^2 = 0.19$, $p = 0.660$; Figures 3B and S2A). These results corroborate earlier studies demonstrating individual recognition in this same northern populations of *P. fuscatus*[41] and demonstrate that a southern

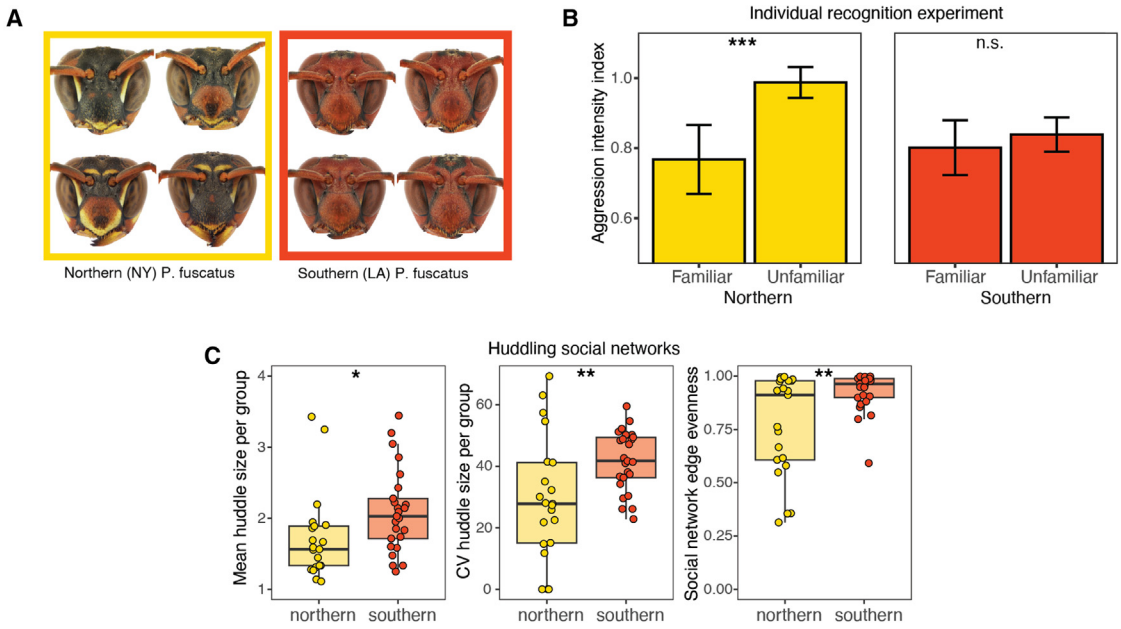



**Figure 3. Social behavior differences between populations with and without identity signals**

(A) Photographs of the faces of wasps from two populations: New York (northern), which have individually distinctive color patterns that function as individual identity signals, and Louisiana (southern), which lack variation in color patterns.

(B) Results from the individual recognition experiment showing less aggression (mean ± SE) between pairs of familiar individuals compared with unfamiliar individuals for northern wasps, but not for southern wasps. Wasps in this experiment encountered unfamiliar individuals on days 1 and 3 and a familiar individual on day 2, who they had previously interacted with on day 0; the results for unfamiliar days are pooled for visualization (n = 164 trials involving 40 northern and 42 southern wasps). See Figure S2A for results separated by day.

(C) Population differences in huddle associations among freely interacting groups of four wasps in a common garden environment (n = 21 northern groups, n = 25 southern groups), showing (left) the mean number of individuals observed "huddling" together each night per group, (middle) the coefficient of variation in huddle size per group, and (right) the edge evenness of social networks derived from huddling behavior. Higher values indicate that connections are relatively evenly distributed among individuals in a network, while lower values indicate more skewed networks with stronger subgroups within the network. Social networks for all groups are shown in Figures S2B and S2C. See also Figure S3.

***p < 0.001, **p < 0.01, *p < 0.05.

population that lacks face diversity also shows no evidence of individual recognition.

We next used the natural difference in recognition ability between northern and southern populations to test the hypothesis that individual recognition stabilizes social networks. To compare social organization among freely interacting groups of four wasps, we characterized nocturnal "huddle" associations, a common behavior exhibited by paper wasps that may facilitate cooperative nesting associations,[39,59,60] in which two or more wasps remain in physical contact or in very close proximity during periods of inactivity. Southern wasps were more gregarious overall but formed fewer stable associations than northern wasps. Southern groups had larger mean huddle sizes than northern wasps ($\chi^2 = 4.52$, $p = 0.033$; Figure 3C), but the coefficients of variation for huddle size through time were greater for southern wasps ($\chi^2 = 7.43$, $p = 0.006$; Figure 3C). This instability was reflected in social networks constructed from huddling associations. Social networks of southern wasps showed relatively even associations among individuals with little apparent sub-structure in the network (Figure S2C). In contrast, networks of northern wasps were often characterized by stronger associations between pairs or trios of individuals to the exclusion of other individuals (Figure S2B). The evenness of interactions among southern wasps was greater than that of northern wasps ($\chi^2 = 9.60$, $p = 0.002$; Figure 3C). Northern wasps thus showed evidence of more stable and individualized social relationships within the groups.

A relatively small number of groups established nests in laboratory enclosures (n = 4 northern groups, n = 7 southern groups) so we report descriptive statistics here rather than statistical tests. Interestingly, the nests of both populations had multiple foundresses, and the mean number of foundresses per nest was similar between populations (northern mean = 2.3, southern mean = 2.2; Figure S3B). However, the foundress associations of southern wasps were less stable through time (Figures S3A and S3C) and their nests grew at slower rates. Northern multi-foundress nests were roughly double the size of southern nests after 2 months (e.g., number of cells, northern mean = 21, southern mean = 10; Figure S3D). These observations come from a small number of nests, and should be interpreted with caution, but are consistent with a role of increased social group stability improving cooperative nesting success.

## Genomic evidence of selection on cognition associated with individual recognition

Previous population genomics studies of northern *P. fuscatus* populations identified multiple strong recent selective sweeps in genomic regions related to learning, memory, and visual

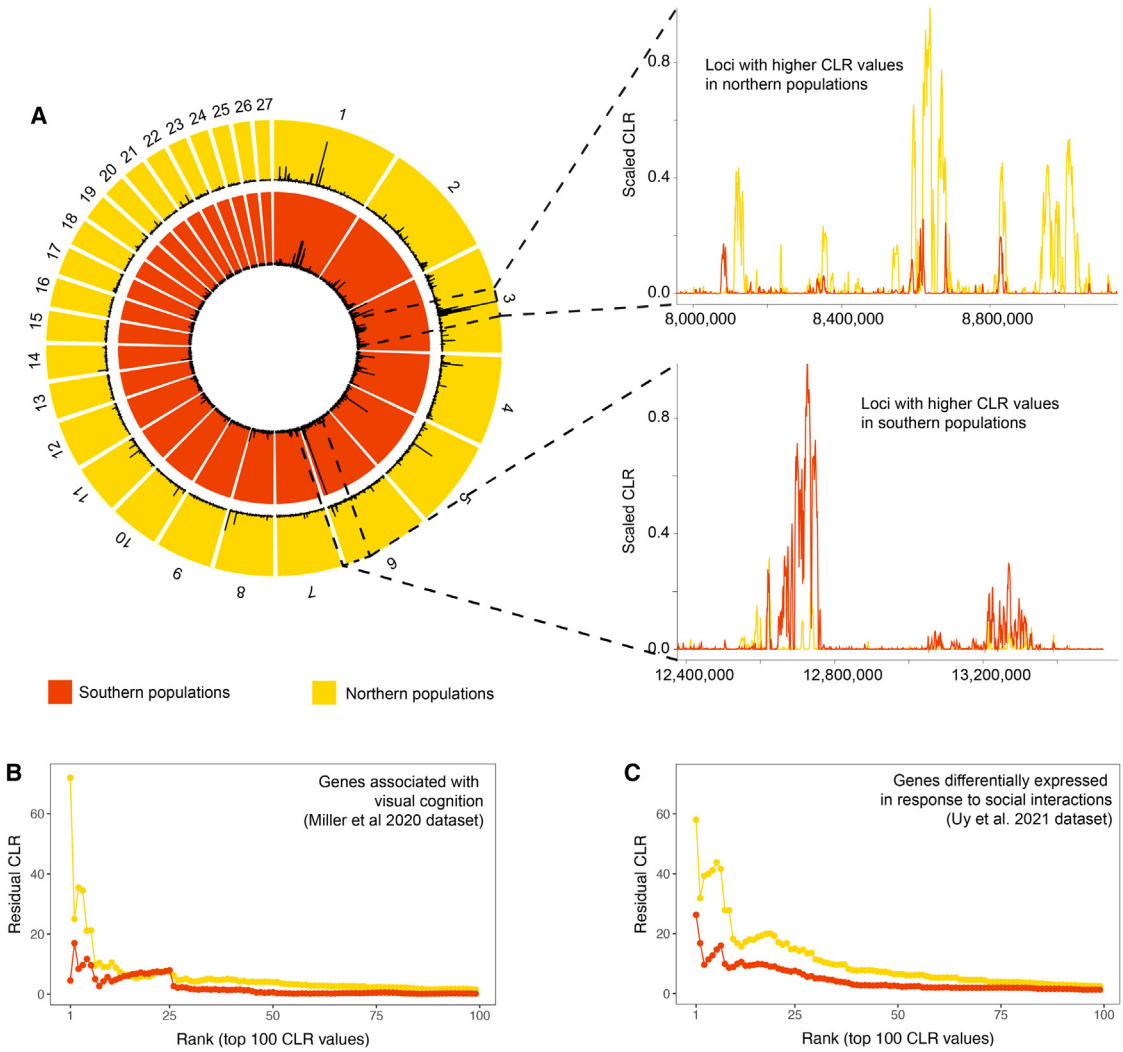



**Figure 4. Stronger and more recent selection on candidate cognition loci in northern populations**

(A) Comparison of scaled composite likelihood ratio (CLR) values between northern (outer) and southern (inner) populations for the largest 27 scaffolds in the *P. fuscatus* genome. CLR values have been smoothed over 10,000 bp windows. Examples of regions where CLR values are greater in the north (top) and south (bottom) are shown.

(B and C) Residual CLR values for the top 100 CLR values of putative social cognition genes from datasets of (B) genes with gene ontology (GO) terms related to learning, memory, and visual processing and (C) genes that are differentially expressed in response to social interactions in northern populations. Both datasets show that northern populations have elevated signatures of selection on putative social cognition genes.

See also Figures S1C and S1D and Tables S1 and S2.

processing, likely related to the recent evolution of individual recognition.[61] We repeated this analysis using southern populations to directly compare evidence of recent selective sweeps between northern and southern populations. Both northern and southern populations show evidence of recent strong positive selection as measured by the composite likelihood ratio (CLR) values from SweepFinder2,[62] with some selective sweeps shared across populations and other selective sweeps that are unique to only one population (Figures 4A, S1C, and S1D). We assessed evidence of selection on loci that likely contribute to cognitive abilities underlying individual recognition using two approaches. First, we compared scaled CLR values between northern and southern populations for loci annotated with gene ontology (GO) terms related to learning, memory, and visual processing, directly replicating the previously published analysis of northern *P. fuscatus* populations.[61] Scaled CLR values for these annotated "visual cognition genes" were elevated in both populations, but there was a significant interaction between population and gene type (gene type, $\chi^2 = 82.43$, $p < 0.001$; population, $\chi^2 = 268.73$, $p < 0.001$; gene type × population, $\chi^2 = 28.50$, $p < 0.001$), indicating that the relative strength of selection on visual cognition genes has been significantly stronger in northern populations than in southern populations (Figure 4B). Second, we compared scaled CLR values between northern and southern populations for genes that are differentially expressed during social interactions in northern *P. fuscatus*.[63] Experimental evidence for differential regulation in response to social interactions suggests that these genes could play a role

in recognition behavior in this species. Again, we find evidence of relatively stronger selection on socially regulated genes in northern compared with southern populations (gene type, $\chi^2$ = 206.56, $p < 0.001$; population, $\chi^2$ = 78.85, $p < 0.001$; gene type × population, $\chi^2$ = 78.69, $p < 0.001$; Figure 4C). Rather than comparing the relative evidence of selection across all genes, we can also ask whether genes in these two datasets are overrepresented among the most strongly selected genes. We find greater enrichment of strongly selected genes in northern compared with southern populations for both GO term and socially regulated gene sets (Table S1). Together, these data show that, compared with southern populations, selection in the north has been relatively stronger on genes that are likely involved in the perceptual and cognitive abilities of wasps to recognize individuals and mediate social interactions.

## DISCUSSION

Social organization and cognitive abilities vary widely among animals. The extent to which they are linked has been a subject of ongoing debate, often involving proxies of both social complexity and cognition. We studied individual recognition, a specific cognitive trait, and its relationship with cooperation. Using three distinct approaches, namely, geographic patterns of cooperation and signal diversity, common garden behavioral assays, and population genomic analyses of selection on cognition loci, we provide cohesive evidence that cooperation favors the evolution of individual recognition. Individual recognition is a bedrock of many complex social behaviors. Our study demonstrates that understanding the factors that shape the evolution of cognitive abilities, such as individual recognition, rather than just brain size or other proxies of general cognition, can provide evidence for a link between social behavior and cognitive evolution. Individual recognition is a complex cognitive trait that involves perception, discrimination, and action components[26,64]; additional studies are needed to identify how specific aspects of cognition have been shaped by selection.

The results of our geographic sampling of color pattern and cooperation are consistent with expectations of selection favoring individuals who signal their identity to facilitate recognition in cooperative populations.[25,47,65,66] The extensive variation in color patterns within and between populations of *P. fuscatus* has long been a source of consternation and puzzlement for students of paper wasps.[67,68] Geographic variation in color patterning is commonly reported in insects and other animals and is frequently linked to selection imposed by the abiotic environment, predation, or sexual selection.[69–74] Our data suggest social selection among female foundresses is the driver of color pattern variation in *P. fuscatus*. Tibbetts et al.[36] also document geographic variation in color pattern diversity in *P. fuscatus* by comparing two populations and show that a population in central mountainous region of Pennsylvania with low pattern diversity also lacks individual recognition. Populations in our dataset come from lower-elevation regions of Pennsylvania at similar latitudes and have similar face diversity to those found further north (Figure 2B), suggesting color pattern diversity can vary over moderate geographic scales. Overall, the data from the broad geographic cline adds to a growing body of research showing that identity information in signals often correlates with measures of social complexity, suggesting that social environments can impose selection on signals to make individuals more recognizable.[75–80]

The results of our common garden studies support the notion that group size and social complexity are not the same.[9,81–83] Initial expectations might be that individual recognition should be associated with larger social groups in general. This pattern is observed in the finding that the number of foundresses per nest and identity signal information covary latitudinally in *P. fuscatus* (Figures 1C and 2B). However, southern wasps actually formed larger huddles, on average, but these huddles were less stable. Social network analysis revealed that northern wasps had stronger relationships among sub-sets of individuals to the exclusion of others, while southern wasps had relatively evenly distributed relationships across the network. This result is consistent with the idea that individual recognition allows for relational social complexity within groups[57,81] and highlights that group size alone may be a poor proxy for social complexity in many contexts.

Our analysis of the genomic data provides an additional lens, suggesting there has been selection on cognitive traits associated with processing social information. Individual recognition appears to be evolutionarily derived and unique to *P. fuscatus* among closely related species.[48,84] Further, population genomic analyses have revealed multiple selective sweeps within the last few thousand years that are enriched for genes likely involved in individual recognition, such as genes related to visual processing, cognition, learning, and memory.[61] Many of these selective sweeps occurred since the last glacial maximum when the Laurentide Ice Sheet covered much of the current northern range of *P. fuscatus*.[85] Together with our results demonstrating that individual recognition and identity signals are absent in southern populations (Figures 2 and 3), these studies suggest a hypothesis in which ancestral populations lacking identity signals and exhibiting low rates of cooperation recently evolved individual recognition as an adaptation to enable successful cooperation as the species expanded northward following the last glacial retreat. The ecological factors that favor cooperation at northern latitudes are currently unknown, but cooperative nesting decreases the probability of nest failure before workers emerge.[38]

### Why do southern populations lack individual recognition?

Given the low population genetic structure at the continental scale of *P. fuscatus*,[50] population differences in color patterning and selection on social cognition suggest multiple possibilities for why we do not observe individual recognition or color pattern diversity in southern populations. First, it may be the case that alleles related to individual recognition arose recently in northern populations and have yet to reach southern populations. Evidence for this scenario comes from a previous analysis of selection in this species that demonstrated that many selective sweeps involved recent *de novo* mutations.[61] However, the lack of population structure suggests that the recent evolution of individual recognition is unlikely to fully explain the geographic pattern of coloration and recognition abilities, as we would expect recognition-associated alleles to quickly spread if they were beneficial in all populations. Indeed, migration of alleles under strong selection in northern populations into southern populations may explain some, though not all, of the shared signatures of selection found

here. Another possibility is that individual recognition is costly in *P. fuscatus*, meaning it is only favorable when rates of cooperation are sufficiently high to make the benefits of recognition outweigh these costs. In particular, the cognitive abilities related to recognition are assumed to be costly in terms of growth and maintenance of the requisite neural tissues.[86–88] Low rates of cooperation in southern populations may then remove the potential benefits of the cognitive mechanisms related to individual recognition, so the alleles for these traits are selected against. Lack of recognition behavior would then also remove benefits of signaling identity via distinctive color patterns. However, models of identity signal evolution suggest that increased signal diversity may be favored, even under very small fitness benefits, provided the costs of distinctiveness are very small or non-existent.[65] Thus, the absence of color pattern diversity in the southern populations suggests that there may be selection either against particular color pattern variants involved in identity signaling or selection favoring the red facial color pattern that is common throughout the Gulf coast region. Future comparative analyses of clinal variation in alleles associated with cognition and color patterning will be useful to help discriminating among the hypotheses raised by the present dataset.

## STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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  - Data and code availability
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  - Photography and color pattern measurement
  - Cooperative nesting data
  - Individual recognition experiment
  - Common garden lab experiment
  - Recent selection in northern versus southern wasps
- QUANTIFICATION AND STATISTICAL ANALYSIS
  - Genomic Data
  - Cooperation and color pattern diversity clines
  - Individual recognition experiment
  - Social network analysis
  - Lab nesting data

## SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2023.11.032.

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## AUTHOR CONTRIBUTIONS

J.P.T. and M.J.S. conceived of and designed the project. J.P.T. and C.M.J. designed and performed the individual recognition experiment. J.P.T. performed other behavioral experiments. J.P.T., C.M.J., S.V., R.J.S., A.W.L., T.J.P., F.M.K.U., and A.W. collected data. S.M.V.B. and H.I.W. developed code for color pattern analysis. J.P.T. analyzed non-genomic data. S.E.M. and M.J.S. analyzed genomic data. M.J.S. secured funding. J.P.T., S.E.M., and M.J.S. wrote the first draft of the paper, and all authors reviewed and edited the paper.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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## STAR★METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Deposited data | | |
| Gene sequence data | This paper | PRJNA761367 |
| Gene sequence data | Miller et al.[61] | PRJNA482994 |
| Wasp cooperation data | This paper, Miller et al.[51] | Dryad (https://doi.org/10.5061/dryad.k3j9kd5f9); https://doi.org/10.1002/ecy.2448 |
| Color pattern data | This paper | Dryad (https://doi.org/10.5061/dryad.k3j9kd5f9). |
| Experimental models: Organisms/strains | | |
| *Polistes fuscatus* paper wasps | Wild populations | N/A |
| Software and algorithms | | |
| Burrow-Wheeler Aligner (v.0.7.13) | Li and Durbin[89] | https://bio-bwa.sourceforge.net/ |
| MICA | Troscianko and Stevens[52] | https://www.empiricalimaging.com |
| ImageJ | Schindelin et al.[90] | https://imagej.nih.gov/ij/ |
| R (version X) | R Core Team[91] | CRAN repository |
| patternize | Van Belleghem et al.[53] | CRAN repository |
| recolorize | Weller[92] | CRAN repository |
| lme4 | Bates et al.[93] | CRAN repository |
| Car | Fox and Weisberg[94] | CRAN repository |
| jackstraw | Chung and Storey[95] | CRAN repository |
| VGAM | Yee[96] | CRAN repository |
| BORIS | Friard and Gamba[97] | https://www.boris.unito.it/ |
| SweepFinder2 | DeGiorgio et al.[62] | http://degiorgiogroup.fau.edu/sf2.html |
| BioCircos | Cui et al.[98] | CRAN repository |
| VCFTools | Danecek et al.[99] | https://vcftools.sourceforge.net/ |
| GATK | Van der Auwera et al.[100] | https://gatk.broadinstitute.org/hc/en-us |
| Analysis code | This paper | Dryad (https://doi.org/10.5061/dryad.k3j9kd5f9). |
| SNPhylo | Lee et al.[101] | https://github.com/thlee/SNPhylo |
| Tassel5 | Glaubitz et al.[102] | https://tassel.bitbucket.io/ |
| Other | | |
| Pro-Kal clear deli cups and lids | TSK Supply (https://www.tsksupply.com/) | 16 oz Clear Punched 50 Count |
| Kritter Keeper | Lee's Aquarium & Pet Products | UPC: 010838200305 |
| Wasp food: waxworms (Galleria mellonella), hornworms (*Manduca sexta*), and mealworms (*Tenebrio molitor*) | Rainbow Mealworms (https://www.rainbowmealworms.com/) | N/A |
| Canon EOS 6D Camera | Canon | SKU:1897C002 |
| Canon 100mm macro lens | Canon | SKU:3554B002 |

### RESOURCE AVAILABILITY

#### Lead contact

Further information and requests should be directed to and will be fulfilled by the lead contact, Michael Sheehan (msheehan@cornell.edu).

#### Materials availability

This study did not generate unique reagents.

### Data and code availability

- New sequence data for samples from Louisiana and Georgia have been deposited to the NCBI Sequence Read Archive. Bioproject accession numbers for samples used in this paper are listed in the key resources table above. SRA IDs for each individual sample are listed in Table S2. All other data used in this paper are publicly available on Dryad as of the date of publication and the DOI is listed in the key resources table.
- All original code has been deposited on Dryad and is publicly available as of the date of publication. The DOI is listed in the key resources table.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

## EXPERIMENTAL MODEL AND SUBJECT DETAILS

### Animals

All animal subjects used in this paper were wild-caught female *Polistes fuscatus* paper wasps. Individuals collected from across the range were used for photography of color patterns and as sources of DNA for genomic analyses. These individuals were captured using nets, freeze-killed, and stored in a -20°C freezer for preservation. GPS coordinates for collection locations are provided in Table S2 and in the Dryad data repository. Individuals used in behavioral experiments were captured in the fall as "gynes" from northern and southern populations and overwintered in the lab. Details on their maintenance and care are provided in the subsections below related to the behavioral experiments ("individual recognition experiment" and "common garden lab experiment").

## METHOD DETAILS

### Genomic analyses

To confirm that northern and southern *P. fuscatus* were the same species, we collected and sequenced the genomes of unrelated female *P. fuscatus* from five populations: New York ($n$ = 30), Massachusetts ($n$ = 10), North Carolina ($n$ = 8), Georgia ($n$ = 15), and Louisiana ($n$ = 25). As an outgroup, we included three individuals each from three closely related species (*P. carolina*, *P. dorsalis*, and *P. metricus*) with sympatric ranges. Sample information is provided in Table S2. Paired-end 150-bp Nextera libraries were sequenced on the Illumina HiSeq 2000. All samples were aligned to the *P. fuscatus* reference genome[61] using the Burrow-Wheeler Aligner (v.0.7.13).[89] Variants were identified using GATK (v3.8)[100] and hard filtered to remove low confidence variants, following the methods described in Bluher et al.[50]

### Photography and color pattern measurement

To photograph faces of wasp specimens, we first removed the head and the antennae to allow full view of the color pattern. We photographed faces under standardized lighting conditions in the lab in a photographic tent using a Canon 6D camera and Canon 100mm macro lens. We confirmed that *P. fuscatus* faces do not reflect light in the ultraviolet range (Figure S4A), therefore standard camera equipment captures the full range of color variation in this species. Specimens were illuminated with bright, diffuse light to minimize shadows and glare by positioning three lights (compact fluorescent) facing away from the specimen to reflect off the walls of the photographic tent and surrounding the specimen with a cylinder of translucent plastic (illumination spectrum provided in Figure S4B). To control for potential slight differences in lighting across days, we also photographed three spectrally flat gray standards (90%, 27%, and 3% reflectance: Color-aid gray set) under identical conditions during each photography session.[52]

Although there is some minor variation in brightness and hue within colors, it is clear to human viewers that the meaningful variation among individuals occurs in patterns of black, red/brown, and yellow (Figures 1 and 2). These three colors are present in most populations of this species and are also the primary colors observed across species of *Polistes*. Therefore, our goal in this analysis was not to measure color *per se*, but to objectively quantify color pattern and compare patterns in homologous regions across individuals. To do so, we first used the MICA toolbox[52] in ImageJ[90] to normalize the light levels across photographs using the gray standards photographed during each session. We then converted these normalized and linearized images using a CIE XYZ cone catch model that was specific to our camera and photography illuminant using the chart-based cone-catch model procedure in the MICA toolbox. We exported these images as .jpg files and adjusted the maximum pixel value to 0.4 out of 1 to make the image appear bright on the screen but without any pixel values being oversaturated.

We then used the R packages *patternize*[53] and *recolorize*[92] to align images, map color patterns, and analyze variation. First, we added 8 landmarks to each face image and then used the 'alignLan' function in *patternize* to align all of the images by these landmarks and mask areas of the image that fell outside of the main regions of interest, encompassing the clypeus, inner eye region, and frons (Figure 2A). Then, we used *recolorize* to classify pixels in these masked images to three color clusters: black, red, and yellow (Figure 2A). To do so, we first obtained a color palette by running an initial color segmentation step on a subset of 30 images that appeared representative of these three colors using the 'histogram' method with 6 bins per color channel using the 'recolorize' function and then implementing the 'recluster' function using a similarity cutoff of 15%. These parameters were chosen based on trial and error to create color segmented images that appeared similar to the color patterns in the original images. We clustered the colors by similarity to three color clusters and took the weighted average of these three clusters which resulted in a color palette corresponding

to the black, red/brown, and yellow present in the images (Figure 2A). We created a separate color palette for the southernmost populations (Louisiana and Georgia) using a different set of 30 images from these populations because these wasps tend to have darker reds than those in more northerly populations. Finally, we classified the pixels of all images to the nearest of these three colors in the palettes using the 'imposeColors' function in *recolorize*. We then converted the images back to rasters consisting of a stack of three binary rasters corresponding to pixel assignments for each of the three colors. Because we were interested in pattern variation, we treated the slightly different black and red colors of the northern and southern wasps as equivalent.

### Cooperative nesting data

We obtained data on the number of foundresses per nest across the latitudinal range of this species using a combination of existing datasets compiled in WASPnest[35,51] and our own observations of nesting behavior. For the WASPnest dataset, we restricted the dataset to observations where the number of foundresses was directly reported. We also excluded observations where the exact number of foundresses were unclear, for example if a paper simply stated that nests were "multi-foundress" without providing the number. We supplemented this dataset with our own observations of foundress associations across the range, including in some key populations at the southern end of the range. We observed nests early in the season before workers emerged. We also observed nests early in the morning or on cool and rainy days when all individuals associated with a nest tend to be on the nest. In total, this dataset consisted of 2,021 nest observations.

### Individual recognition experiment

Our experimental design generally followed previous studies of individual recognition in *P. fuscatus* and other social insects.[36,42,48,58] We compared aggression between pairs of familiar versus unfamiliar wasps in neutral arenas, while controlling for potential changes in aggression across days that are unrelated to the familiarity of the two wasps. We used lab overwintered *P. fuscatus* gynes that were collected in the fall of 2019, from northern (NY and ME) and southern (LA) populations. Individuals were overwintered with their nestmates in plastic deli cups, and provided water and sugar, as well as crumpled construction paper in which to hide. They were overwintered for approximately three months at 4°C for northern wasps and 10°C for southern wasps, to account for natural differences in winter temperatures between these populations. Following overwintering, wasps were weighed, marked with paint on their thorax (Testors enamel paint), and housed individually in deli cups for 5–6 days before the start of the experiment at a temperature of approximately 23°C with 12:12 light–dark cycle.

Separately for each population, we ranked individuals by weight to create three weight classes of similarly sized individuals. We then paired individuals together such that they always encountered other individuals from different nests but from the same weight class. These criteria resulted in 40 northern and 42 southern wasps for the experiment. On Day 0, pairs of wasps were placed in plastic petri dishes and filmed for 45 mins. Immediately following this trial, the pair was housed together in a new deli cup overnight to give the individuals additional time to become familiar with each other. Between 9 and 10 AM the next morning (Day 1) these paired wasps were then put into solitary housing where they remained for the rest of the experiment other than during trials. On Day 1 and 3 of the experiment, wasps were paired and filmed interacting as described above but with new individuals they had never encountered before. On Day 2 of the experiment, they were paired again with the same individual they interacted with on Day 0. We additionally controlled for potential day effects by starting the experiment for half of the wasps on one day and the other half on the subsequent day. All interaction trials occurred during the afternoon (13:00-18:00) at temperatures ranging from 25 to 26°C.

We scored aggressive behaviors for the first 15 minutes of each trial using BORIS.[97] Our ethogram was developed based on a combination of established ethograms for *Polistes*,[39] and our own preliminary observations of the aggressive behaviors that are common in this type of experiment. We scored the following as point behaviors (instantaneous behaviors that are counted for each occurrence): dart, a rapid forward movement towards another individual; snap, open mandibles towards another individual; bite, mandibles closing on another individual; kick, rapid leg extension that appeared to push off or push away another individual. We scored the following as state behaviors (behaviors that have durations): chase, one wasp pursuing another wasp who appears to be avoiding the interaction; antennation, probing another individual with antennae; grapple, wrestling-type behaviors with both individuals engaged with biting and kicking; huddle, two wasps in close proximity without interacting aggressively. Observers were blind to treatments and experiment day when scoring behaviors.

For each trial ($n$ = 164), we summed the total numbers of point behaviors, and summed the durations of all state behaviors. For analyses, we converted the durations of state behaviors into point events with one second duration equal to one observation of a behavior. We computed an aggression intensity index, similar to Sheehan and Tibbetts.[42,48,58] Specifically, aggressive behaviors were weighted on a scale from 0 to 4, with higher scores indicating behaviors characteristic of more escalated aggressive interactions. These weights were: (0) huddling, (1) antennation, chase, dart, kick, dodge, (2) snap, (3) bite, (4) grapple. We summed these weighted behaviors and divided by the total number of behaviors to compute an aggression intensity index.

### Common garden lab experiment

Lab overwintered wasps from were individually marked and housed in groups of four individuals: three individuals from one nest of origin and another individual from a different nest. This design was meant to mimic common foundress associations, with co-foundresses often being relatives but with occasional non-relatives joining foundress associations. We performed this experiment in the spring of 2020 (n = 10 northern groups and 13 southern groups) and again in the spring of 2022 (n = 11 northern groups and 12 southern groups). Groups of wasps were housed in enclosures consisting of two 36.8 cm × 22.2 cm × 24.8 plastic Kritter Keepers (Lee's

Aquarium & Pet Products) stacked on top of each other, with ventilation holes drilled into the sides and top. Four 10 cm x 10 cm cardboard nesting "huts" were attached to the top of the enclosure to provide each wasp the option to either nest alone or co-found a nest with other individuals. Each enclosure was provided with ample crumpled cardboard paper to provide nesting material, as well as a sugar cube, honey, water, and, once nests were established, an *ad libitum* variety of larval insects (waxworms (*Galleria mellonella*), hornworms (*Manduca sexta*), and mealworms (*Tenebrio molitor*); Rainbow Mealworms). Wasps were kept in a temperature-controlled room under conditions meant to mimic warm summertime environments to stimulate nesting (14:10 light-dark cycle, 25-28°C daytime temperature, 21-25°C nighttime temperature, 20-40% humidity).

Before the lights came on each morning, we recorded the location of each individual relative to other individuals in the group as either: alone – greater than one body length from any other individual; in proximity – within one body length of another individual; or huddled – touching or close enough to be capable of touching another individual. Once a nest was established in an enclosure, we also recorded which individuals were on or next to the nest overnight for the duration of the experiment. Individuals often leave the nest to forage or acquire nesting materials during the day but return and remain on the nest at night.[35,39] Therefore, nighttime surveys provide a reliable measure of which individuals are associated with the nest. We measured nest development of all nests two months after housing by counting the number of cells in the nest and weighing the nest as well as any emerged workers or males. Other metrics of nest development, such as computing growth rates using the time since nest establishment as the denominator, produced similar results.

### Recent selection in northern versus southern wasps

Using the 40 re-sequenced *P. fuscatus* genomes from Georgia and Louisiana, we looked for evidence of selective sweeps in southern wasps with SweepFinder2.[62] SweepFinder2 uses deviations in the local site frequency spectrum to infer selective sweeps, generating a composite likelihood ratio (CLR) value for each window. Larger CLR values provide evidence of stronger selection, more recent selection, selection on newer mutations, or some combination of these phenomena.[61] We compared CLR values for the southern population to CLR values that were generated for a prior study of northern populations.[61] Northern CLR values were calculated from the same 40 wasps from New York and Massachusetts described above. We included two sampling sites in each analysis to avoid detecting selective sweeps caused by local adaptation. Because estimates of CLR values can be influenced by other population parameters, such as effective population size, we scaled CLR values for each population separately to the maximum CLR value in each dataset. Values were compared in 1000 bp windows across the genome and plots were constructed with BioCircos.[98] For each gene in the genome, as well as the region +/- 5000 bp upstream/downstream of that gene, we calculated a maximum scaled CLR value.

Genes in the *P. fuscatus* genome had been previously classified as potential targets of selection for cognitive evolution if annotated with one of the following Gene Ontology (GO) terms: cognition (GO:0050890), mushroom body development (GO:0016319), visual behavior (GO:0007632), learning or memory (GO:0007611), and eye development (GO:0001654). Out of 11,935 genes, 1,088 genes were considered potentially related to the perceptual and cognitive mechanisms of individual recognition (hereafter: 'visual cognition genes'). We also categorized genes based on whether or not they showed evidence of differential expression in response to social experience based on data published in Uy et al.[63]

## QUANTIFICATION AND STATISTICAL ANALYSIS

All statistical analyses were conducted in R version 4.1.3 (2022-03-10).[91]

### Genomic Data

To examine the evolutionary relationship between samples of *P. fuscatus* collected from different populations (Figure 1A), we constructed a phylogenetic tree with SNPhylo (v20160204),[101] a program designed to rapidly build phylogenetic trees from large SNP datasets. To reduce the size of the dataset, variants were first filtered with VCFtools[99] to retain only a single, informative, high-quality, biallelic SNP every 1,000 bp using the options: –max-alleles 2 –mac 0.1 –max-missing-count 10 –min-meanDP 3 –max-meanDP 1200 –minQ 20 –thin 1000. SNPhylo was run with 500 rounds of bootstrapping. The phylogeny is shown in Figure 1B. We further explored relatedness between samples by conducting a PCA of genetic variants using Tassel5[102] (Figures S1 and S2). Lastly, we calculated genetic differentiation between the most distant populations, New York (n=30 sequenced wasps) and Louisiana (n=25 sequenced wasps), using Weir-Cockerham FST, implemented in VCFtools.

To statistically compare scaled CLR values between populations and gene categories, we log transformed scaled CLR values to improve linearity and fit linear mixed effects models using the *lme4* package, with population (northern or southern), gene type (GO term dataset: visual cognition gene or other; differential expression dataset: yes or no), and their interaction as fixed effects, and gene identity as a random effect. We evaluated the significance of fixed effects and their interaction using type III ANOVAs using the *car* package, and we report Wald chi-square test statistics. We visualized population-specific elevation of CLR values for candidate social cognition loci by computing the residual CLR value per locus. To do this, we generated expected CLR values by randomly selecting 100 sets of n non-candidate loci, where n is the number of candidate loci for a dataset, i.e., n = 1,088 genes based on visual cognition GO terms, n = 733 genes for socially regulated genes. We then ranked each set by decreasing CLR value and took the mean CLR value at each rank across the 100 sets to estimate expected CLR values for n random loci.[103] We also ranked the observed CLR values for candidate loci and took the difference between the observed CLR value and expected CLR value for each rank as the residual CLR. These residuals thus control for potential population differences in CLR values across the genome and allow visualization of potential differences in the elevation of CLR values for candidate loci.

### Cooperation and color pattern diversity clines

For the cooperative nesting data across the geographic cline, we analyzed the relationship between the number of foundresses per nest and latitude (n = 2,021 nest observations) using a zero-truncated Poisson regression using the *VGAM* package.[96] For the color pattern diversity cline, we then analyzed variation among face patterns using the raster stacks with each of three raster layers per image corresponding to one color. We used *patternize* to compute a principal components analysis of these rasters which yielded 267 components corresponding to the 267 images in the data set. We reduced this dataset to 23 statistically significant components (Table S3), which were determined using permutation parallel analysis in the *jackstraw* package.[95] We then computed pairwise Euclidian distances between points in this multi-dimensional PCA space and quantified within-site face diversity as the mean pairwise distance between points collected from the same site (Figures 2A and 2B). The sites and number of wasps per site were as follows: Mandeville, LA (n = 17); Franklinton, LA (n = 12); Brunswick, GA (n = 5); Savannah, GA (n = 6); Greenville, SC (n = 32); Gastonia, NC (n = 17); Durham, NC (n = 9); Hollister, NC (n = 6); Petersburg, VA (n = 16); Baltimore, MD (n = 6); Hanover, PA (n = 11); Linesville, PA (n = 28); Ames, IA (n = 18); Binghamton, NY (n = 16); Ithaca, NY (n = 35); Sanford, ME (n = 5); Lake Pleasant, NY (n = 24); Brandon, VT (n = 6). We statistically analyzed the relationship between latitude and face diversity using linear regression.

### Individual recognition experiment

For the individual recognition experiment we compared this aggression intensity index between pairs of 'familiar' wasps (Day 2) and 'unfamiliar' wasps (Days 1 and 3) and these pooled data are shown in Figure 3B. Data for all experiment days are shown in Figure S2. In total we conducted 164 behavioral trials (n=80 in northern wasps,20/day; and n=84 in southern wasps, 21/day) including a total of 82 wasps (n=40 northern and n=42 southern wasps). Separately for each population, we fit linear mixed effects models of the aggression intensity index using the *lme4* package,[93] with treatment ('familiar' vs. 'unfamiliar') as a fixed effect, and with experiment day, cohort, and individual as random effects. Significance of the main effect of treatment was evaluated using Wald chi-square tests implemented through the *car* package.[94]

### Social network analysis

We analyzed pre-nesting associations for the first two weeks of the experiment because all nests were established by two weeks into the experiment. For groups that did not build a nest, we used the full two weeks of data. For groups that built a nest, we only used data from before the nest was established. Similarly, 6 individuals from 6 different groups died during the first two weeks of the experiment, so for these groups we also only used data from before one individual in the group died. To compute descriptive statistics of the number of individuals per huddle (huddle size), we first computed the mean huddle size per group-per day, and then used these numbers to compute grand mean and coefficients of variation for each group.

We also used the pre-nesting huddle data to construct social networks for each group. Connections between individuals (''edges'') were weighted depending on whether individuals were huddled together (weight = 2) or simply in proximity (weight = 1). From these social networks, we computed what we define here as ''edge evenness''. Analogous to species evenness in ecology,[104] edge evenness describes how evenly distributed relationships are across the network. Networks in which individuals interact at similar rates with all other individuals in the network have higher edge evenness than those in which some pairs or trios of individuals have stronger relationships than others. Edge evenness ($J'$) was computed as

$$J' = \frac{H'}{\ln(S)}$$

where $S$ is the number of possible edges in the network, in our case 6 for a 4-individual network, and $H'$ is the Shannon diversity index

$$H' = -\sum_{i=1}^{S} p_i \ln(p_i)$$

where $p_i$ is the proportion of weight of the $i$th edge in the network relative to the sum of all weights in the network. Edge evenness describes how evenly distributed edge weights are across the network. Networks in which individuals interact at the same rates with all other individuals in the network have an edge evenness of 1, while lower values indicate skewed networks in which some pairs or trios of individuals have stronger relationships than others.

We statistically compared populations in terms of their mean and coefficient of variation in huddle size, as well as social network edge evenness, by fitting linear mixed effects models of using using the *lme4* package,[93] with population (northern vs southern) as a fixed effect and year as a random effect. Significance was evaluated using Wald chi-square tests (*car* package[94]).

### Lab nesting data

For groups that started nests in the lab, we report the mean number of foundresses observed on a nest for the first 30 days since nest establishment. We also report a measure of instability in foundress associations that sums the number of times there was a change in who was on the nest from the previous night, divided by the number of days. Because of the small sample size of numbers of nests, we only report descriptive statistics of foundress associations and nest development.