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ARTICLE

Genetic and environmental effects on morphological traits of social phenotypes in wasps

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Many species exhibit distinct phenotypic classes, such as sexes in dioecious species or castes in social species. The evolution of these classes is affected by the genetic architecture governing traits shared between phenotypes. However, estimates of the genetic and environmental factors contributing to phenotypic variation in distinct classes have rarely been examined. We studied the genetic architecture underlying morphological traits in phenotypic classes in the social wasp *Vespula maculifrons*. Our data revealed patriline effects on a few traits, indicating weak genetic influences on caste phenotypic variation. Interestingly, traits exhibited higher heritability in queens than workers. This result suggests that genetic variation has a stronger influence on trait variation in the queen caste than the worker caste, which is unexpected because queens typically experience direct selection. Moreover, estimates of heritability for traits were correlated between the castes, indicating that variability in trait size was governed by similar genetic architecture in the two castes. However, we failed to find evidence for a significant relationship between caste dimorphism and caste correlation, as would be expected if trait evolution was constrained by intralocus genetic conflict. Our analyses also uncovered variation in the allometric relationships for traits. These analyses suggested that worker traits were proportionally smaller than queen traits for most traits examined. Overall, our data provide evidence for a strong environmental and moderate genetic basis of trait variation among castes. Moreover, our results suggest that selection previously operated on caste phenotype in this species, and phenotypic variation is now governed primarily by environmental differences.

Heredity (2024) 133:126–136; https://doi.org/10.1038/s41437-024-00701-5

INTRODUCTION

Individuals within species display remarkable phenotypic diversity. In many cases, different phenotypes can develop from the same genome (Yang and Pospisilik 2019; Yoon et al. 2023). Such developmental plasticity is critical to allowing organisms to adapt to their environment and produce forms well suited for contemporary conditions (West-Eberhard 2003; Yoon et al. 2023). Eusocial animals represent a fascinating and important group to study the evolution of phenotypic diversity (Wilson 1971; Szathmáry and Smith 1995). Highly social species, such as the social insects, which include ants, termites, and some bees, wasps, thrips, aphids, and beetles, thrive in colonies made up of phenotypically distinct individuals with unique functional roles (Oster and Wilson 1978). Food acquisition, colony defense, reproduction, and brood care are executed by distinct caste members in a cooperative manner (Oster and Wilson 1978; Wilson and Hölldobler 2005).

The caste system found in social insect taxa is particularly interesting because multiple phenotypes often arise from a single genotype (Wilson 1971; West-Eberhard 2003; Taylor et al. 2019). Social insect castes can be divided into reproductive and nonreproductive individuals, which often differ dramatically in morphological, behavioral, and physiological traits (Wilson 1971; Treanore et al. 2021). For example, reproductive ant queens reproduce and disperse aerially whereas workers are typically incapable of mating or flight. Similarly, termite reproductive castes have wings to disperse and eyes to view their environment, whereas sterile workers and soldiers usually do not. In addition to striking trait differences, the reproductive and nonreproductive castes also typically differ dramatically in size (Treanore et al. 2021; Shingleton and Vea 2023). The reproductive castes of virtually all social insects are larger than the worker and soldier castes (Wilson 1971). On a proximate level, these size differences arise, in part, from different development regimes that the castes experience. Ultimately, the size differences represent adaptations related to the function of the individual castes that benefit the colony overall.

The sharing of a common genome between reproductive and nonreproductive social insect castes presents interesting opportunities to study how a single genome can produce multiple distinct phenotypes (Linksvayer and Wade 2005; Pennell et al. 2018). For instance, differential selection on castes occurs when the optimal phenotype for a particular trait differs between queens and workers (Holman et al. 2013; Holman 2014). Indeed, we expect there to be selection on a variety of different traits between castes because of the dramatic differences in behavior and functionality that castes display. Consequently, social insects serve as attractive systems for understanding general aspects of genetics and development because selection acting in opposing directions can lead to evolutionary conflict between the castes (Szathmáry and Smith 1995; Mank 2017).

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Received: 27 September 2023 Revised: 14 June 2024 Accepted: 17 June 2024

The goal of this study was to examine genetic and environmental effects on the development of social phenotypes in the Eastern Yellowjacket wasp, *Vespula maculifrons*. *V. maculifrons* is a ground-dwelling, social wasp commonly found in eastern North America and has distinct worker and queen castes (MacDonald and Matthews 1981). However, social wasp castes are not as strongly differentiated as, for example, ant and termite castes. Further, the behavioral repertoires of wasp castes are not as divergent as those for queens and workers in ants, where workers are flightless and queens in claustrally founding species do not participate in foraging. Thus, *Vespula* wasps represent an interesting taxon to investigate genetic conflict because they show modest caste differences. Moreover, as with most *Vespula* wasps, *V. maculifrons* colonies are headed by a single, polyandrous queen (i.e., the queen mates with multiple males) (Foster and Ratnieks 2001; Goodisman et al. 2007b; Kovacs et al. 2008; Dyson et al. 2021). The resulting social structure provides a strong model system to study the effects of genotype on the formation of polyphenisms and the origin of genetic conflict (Taylor et al. 2018; Lester and Beggs 2019).

Here, we investigated the effects of genotype and the environment on the morphology of *V. maculifrons* queen and worker castes (Fig. 1A). Our primary interest in this study was to understand genetic and environmental effects operating on morphological traits in *V. maculifrons*. Thus, we estimated levels of narrow-sense heritability for a series of morphological traits in both queens and workers. We were interested in understanding the magnitude of heritability estimates within castes, as well as how heritability estimates varied between castes. We also investigated if genetic conflict might operate on morphological traits by examining relationships between trait dimorphism and genetic effects operating on individual traits. Finally, we examined allometric scaling relationships in the queen and worker castes to determine if selection pressures might operate differently in each caste (Wilson 1953; Nijhout and Wheeler 1996; Kovacs et al. 2010a, 2010b). Overall, this study improves our current understanding of the role of genetic and environmental effects in the development of social phenotypes. This investigation also provides insight into the influence of genetic conflict on the evolution of distinct phenotypic classes within species.

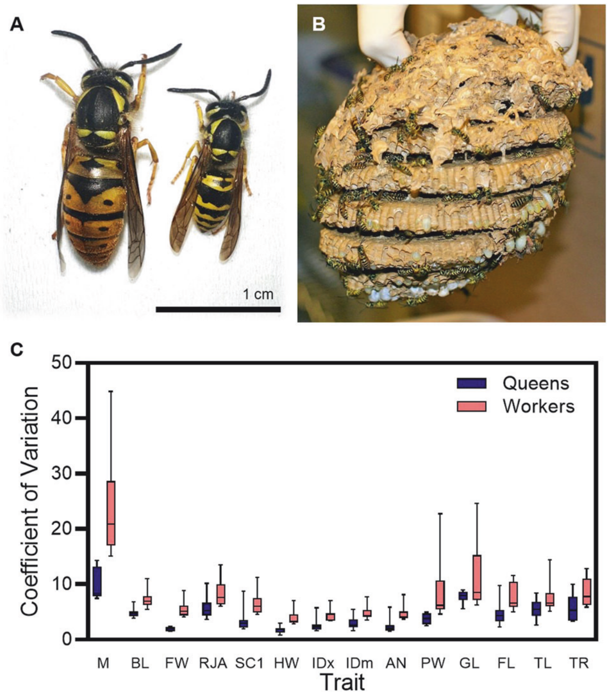

Fig. 1 The Eastern Yellowjacket Wasp, *Vespula maculifrons*, and morphological variability among queen and worker castes. **A** Queen (left) and worker (right) castes of *Vespula maculifrons*. **B** A small, mature *V. maculifrons* colony. **C** Mean coefficients of variation for all 14 measured traits were larger in workers than queens. Box plot whiskers represent the 5th and 95th percentile among colonies. Traits are abbreviated as M mass, BL body length, FW forewing length, RJA reslin joint area length, SC1 submarginal cell 1 length, HW head width, IDx maximum interorbital distance, IDm minimum interorbital distance, AN antenna length, PW pronotum width, GL second segment gaster length, FL femur length, TL tibia length, and TR tarsus length.

METHODS

Wasp collection

We collected 10 mature colonies of *V. maculifrons* wasps from a single interbreeding population (Hoffman et al. 2008) around the Atlanta, Georgia, USA area during early November 2021 and 2022. Collection at this time of the year ensured that colonies would contain abundant numbers of prereproductive queens (gynes; hereafter used interchangeably with 'queens') and workers (Fig. 1A, B, Supplementary Table S1). Briefly, ether was poured into the entrance hole to temporarily anesthetize wasps, and nests were dug out and placed in a ventilated plastic container. Wasp colonies were brought back to lab, euthanized via rapid cooling, and stored at −20 °C. We sorted 100 workers and 40–50 queens into sterile microcentrifuge tubes for further analysis.

Genetic analysis

We removed a single posterior leg from each wasp into a separate, sterile tube for DNA extraction using the Chelex method (Walsh et al. 1991). Briefly, we ground tissue with clean pestle in liquid nitrogen and added 250 µL of 5% Chelex solution. Samples were vortexed and heated on a 95 °C block for 30 min. Samples were stored at −20 °C and vortexed and centrifuged before use.

Individuals were genotyped at six highly variable DNA microsatellite markers (Foster et al. 2001; Daly et al. 2002; Hasegawa and Takahashi 2002; Dyson et al. 2022) and amplified via PCR in duplex reactions as previously described (Dyson et al. 2022, Orr et al. 2024) (Supplementary Table S2). Successful PCR amplification was confirmed via visualization of DNA bands on a 3% agarose gel with 1% ethidium bromide. PCR products of all six microsatellites were combined in equal ratios with a small amount of ROX-labeled ladder and formamide. We heated samples to 96 °C for 3 min to ensure denaturation and then cooled samples on ice for 1 min. Samples were mailed overnight in 96-well plates to Eton Biosciences for fragment analysis. Allele sizes were manually recorded using ThermoFisher Connect$^{\text{TM}}$ Microsatellite Analysis software. Then, queens and workers within colonies were assigned to distinct patrilines based on the multilocus genotype distributions and consideration of the haplodiploid genetic system displayed by *V. maculifrons* (Goodisman et al. 2007a).

We estimated the allele frequencies of each locus. We then calculated the non-detection error for the combined genetic information. The nondetection error is the probability that two males would share the same multilocus genotype and, therefore, be undetected as separate mates of a focal queen in our genetic analyses (Boomsma and Ratnieks 1997). We found that our nondetection error was 0.000249. Therefore, using these six microsatellite markers, we can assign patriline of workers and queens within colonies with high confidence.

Morphological measurements of queens and workers

We obtained the wet mass of each individual with Mettler Toledo ABI35-S scale. We then determined the size of a suite of 13 morphological traits of each wasp. Traits were chosen based on previous morphometric analysis from literature in other hymenopteran insects (Radloff et al. 2005; Kovacs et al. 2010a, 2010b; Alattal et al. 2014) and included: body mass (M), body length (BL), forewing length (FW), reslin joint area length (RJA), submarginal cell 1 length (SC1), head width (HW), maximum interorbital distance (IDx), minimum interorbital distance (IDm), antenna length (AN), pronotum width (PW), second gaster segment length (GL), femur length (FL), tibia length (TL), and tarsus length (TR) (Supplementary Fig. S1).

Wasps were individually dissected and measured using an Olympus SZX16 dissecting microscope and Teledyne Lumenera Infinity5 camera software. Only three of the most frequently genotyped patrilines were phenotyped to maximize statistical power. All individuals within a single colony were measured by the same researcher to minimize within-colony observer measurement bias. There was a total of four researchers that participated in measuring, and all measurements were performed blinded (i.e., without knowing patriline information). We also examined if individual researchers varied in how they measured traits. To investigate this issue, two distinct researchers measured the same traits (FW, RJA, SC1) on a subset of wasps from five colonies. Notably, we found highly significant ($P < 0.005$) and positive Spearman's rank correlations for all traits in both workers and gynes, suggesting that observer measurement differences were minimal.

Data analysis

All statistical analyses were performed in JMP Pro 16 software. We performed principal component analysis on the traits to obtain and analyze size-associated variables that were independent of each other (Supplementary Fig. S2). We then included principal components 1, 2, and 3 as variables in subsequent analyses.

Genotypic data were analyzed to estimate queen mate number (K) (Goodisman et al. 2007b). Coefficients of variation of traits were calculated by dividing the standard deviations by the means for each trait and caste. Dimorphism (D_i) of traits between castes was calculated as $D_i = 1 - (\overline{X}_i^Q/\overline{X}_{bl}^Q)/(\overline{X}_i^W/\overline{X}_{bl}^W)$, where $\overline{X}$ denotes the sample mean, i denotes a particular trait, bl denotes body length, Q denotes queens and W denotes workers (Kovacs et al. 2010a, 2010b). The calculated D_i values were estimated for each of the ten colonies separately and then averaged together to obtain a single value per trait. D_i statistics that are positive indicate that queen traits are larger than worker traits at the same mass. Alternatively, D_i statistics that are negative would indicate that workers traits are larger than queen traits at the same mass. Finally, a D_i statistic of 0 represents monomorphism, or phenotypically indistinguishable castes.

We were also interested in understanding the allometric scaling relationships for queens and workers. These scaling relationships demonstrate how different traits contrast between castes and can provide insight into evolutionary pressures affecting trait evolution. Body mass was chosen as our size metric to most accurately represent the overall size of each individual (Hallgrímsson et al. 2019). Therefore, scaling relationships were determined by plotting natural log trait size against natural log body mass (Glazier 2021) and performing a linear regression for each caste. Differences in scaling relationships between castes were determined using an ANOVA F-Test to determine if the linear allometric scaling slopes differed between castes.

We next investigated if genetic variation affected trait variation within each caste. First, we normalized all raw data by taking the natural log of each measure (Glazier 2021). We then used a nested random effects model with two random-effects variables, colony and patriline (nested within colony), to determine if genetic variation influences trait variation (Borenstein et al. 2010). We then investigated if there were statistically significant genetic effects on trait size for each caste by running a Wald test on our model (Wald 1945).

We estimated narrow-sense heritability (h^2, defined from 0 to 1) for all traits using the "animal model" approach with the R package "MCMCglmm" to fit a Bayesian mixed model to the trait data (Hadfield 2010). Narrow-sense heritability (h^2) quantifies the proportion of phenotypic variance (V_p) that is attributable to additive genetic variance (V_A), which is the variance due to the sum of the average effects of individual alleles. We used the makeS function in the nadiv package in R to create an inverse genetic relatedness matrix that treated all traits as sex-linked in order to account for haplodiploidy (Wolak 2012). Specifically, we modeled individual ID and maternal effects (colony) as random effects, with the genetic relatedness matrix (inverse) being used to model the additive genetic variances. We used weakly informative priors for 250,000 iterations with a burn-in of 50,000 and a thinning interval of 100 (full R script is included in the Dryad Digital Repository). This approach, therefore, estimates narrow-sense heritability by considering the proportion of phenotypic variance explained by the additive genetic variance captured through the pedigree-based relatedness matrix.

We next determined if patrilines exerted similar effects in queens and workers. That is, we were interested in understanding if a particular patriline that produced large (small) trait size in workers also produced large (small) traits size in queens. Such a finding would indicate that genotypic influences on trait size were correlated between castes. To investigate this question, we first calculated the linear correlation (R value) between queen and worker traits arising from the three different patrilines assayed within each colony ($n = 3$). We then took the mean of the correlations for all colonies to produce a single estimate of the 'within colony caste correlation' (WCCC) for each trait. Positive WCCCs (closer to 1) indicated that patrilines that produced larger (smaller) queen traits also produced larger (smaller) worker traits. In contrast, negative WCCCs (closer to −1) indicated that patrilines that produced larger (smaller) queen traits tended to produce smaller (larger) worker traits. We performed an ANOVA to generate a p-value for each individual colony's caste correlation. Then, the overall significance of the WCCC for each trait was determined by combining each colony's p-value using Stouffer's method.

We also calculated a caste correlation for each trait by treating all patrilines as independent from colony ($n = 30$ patrilines). This 'across colony caste correlation' (ACCC) considers the effects of patriline within colony on trait size, like the WCCC. However, unlike the WCCC which effectively controls for differences in trait size among colonies, the ACCC deliberately includes information on differences in trait size among colonies. Positive ACCCs (closer to 1) indicated that colonies that produced larger (smaller) queen traits also produced larger (smaller) worker traits. In contrast, negative ACCCs (closer to −1) indicated that colonies that produced larger (smaller) queen traits tended to produce smaller (larger) worker traits. The significance of the ACCC for each trait was determined by performing an ANOVA test on the linear regression of each colony's mean queen and worker trait measurement ($n = 10$). Notably, significant ACCC can arise from either genetic or environmental influences on trait size whereas significant WCCC can arise from genetic influences on trait size only.

Finally, we investigated if there was a relationship between trait caste dimorphism (D_i) and the WCCC or ACCC. Such a relationship is expected if traits that show low caste correlations are able to freely evolve dimorphic phenotypes (Cox and Calsbeek 2009; Wyman et al. 2013). One would expect a particular trait that evolves strong dimorphism to be relatively free of conflict arising from caste correlations.

RESULTS

Morphological differences between castes

All morphological traits differed significantly in size between the queen and worker castes. As expected, queen traits were always larger than worker traits in all colonies ($P < 0.0001$). Of greater interest, however, was that worker traits showed greater size variation than queen traits (Fig. 1C). In all cases, the worker coefficients of variation were larger than those of queens, which indicates that workers are more variable for their size than queens. We also calculated caste dimorphism (D_i) for each trait. We found dimorphism values were relatively small and similar for most traits (Table 1). However, notably, dimorphism values were always positive indicating that queen traits were always larger than worker traits when standardized for body size. For example, the largest dimorphism value was 0.2194 for antenna length, which suggests that queens would have 21.94% longer antenna than workers of the same size.

The size of traits within individuals are likely to be strongly correlated with each other. Consequently, we used principal component analysis (PCA) to generate new principal component variables that would be uncorrelated to each other for analysis. We found that 87.1% of variation within our dataset was explained by principal component (PC) 1 with an eigenvalue of 12.19. All traits loaded strongly (~0.9) and positively on PC1 (Fig. 2). PC2 had an eigenvalue of 0.483 and explained 3.5% of the variance. Some traits loaded negatively on PC2 (e.g., gaster length) and others loaded positively (e.g., pronotum width). Finally, we included PC3 in our analyses. PC3 had an eigenvalue of 0.289 and explained 2.1% of the variance. PC3 also had traits loading both positively and negatively on it.

Scaling relationships of castes

We examined patterns of allometry for queen and worker traits by examining the relationship between trait size and body size in the queen and worker castes. All traits scaled positively with body mass (Fig. 3). Most traits exhibited a linear, nearly parallel

Table 1. Caste dimorphism, statistical significance of patriline (genetic) effects, and statistical significance of colony (genetic + environmental) effects for queen and worker traits.

Trait	Symbol	Dimorphism	Queen patriline effects *P* value	Worker patriline effects *P* value	Queen colony effects *P* value	Worker colony effects *P* value
Body mass	M	–	0.3586	0.525	0.0776	**0.0437**
Body length	BL	–	0.1733	0.791	0.0807	**0.049**
Forewing length	FW	0.149	0.1979	0.2405	0.1228	0.0709
Reslin joint area length	RJA	0.1205	**0.0223**	**0.036**	**0.0484**	**0.0437**
Submarginal cell 1 length	SC1	0.1407	0.0657	**0.0284**	0.2535	0.1199
Head width	HW	0.1795	0.08	0.5335	0.0914	0.0623
Interorbital distance maximum	IDx	0.1884	0.4624	0.3895	0.059	0.0521
Interorbital distance minimum	IDm	0.1655	0.2354	0.8863	0.058	**0.0455**
Antenna length	AN	0.2194	0.7822	0.4403	0.0501	**0.0462**
Pronotum width	PW	0.0553	0.2504	0.3757	**0.0352**	**0.038**
Gaster segment length	GL	0.037	0.7134	0.5148	**0.0499**	**0.0384**
Femur length	FL	0.1086	0.3064	0.1928	0.1059	0.0574
Tibia length	TL	0.1448	0.9564	0.0611	0.0582	0.1013
Tarsus length	TR	0.1013	0.6537	0.5813	**0.0471**	**0.0432**
Principal component 1	PC1	–	0.1906	0.8662	**0.047**	**0.0497**
Principal component 2	PC2	–	0.7877	0.9078	**0.0363**	**0.0368**
Principal component 3	PC3	–	**0.0435**	0.0656	**0.0435**	**0.0393**

Statistically significant *P*-values are shown in bold font.

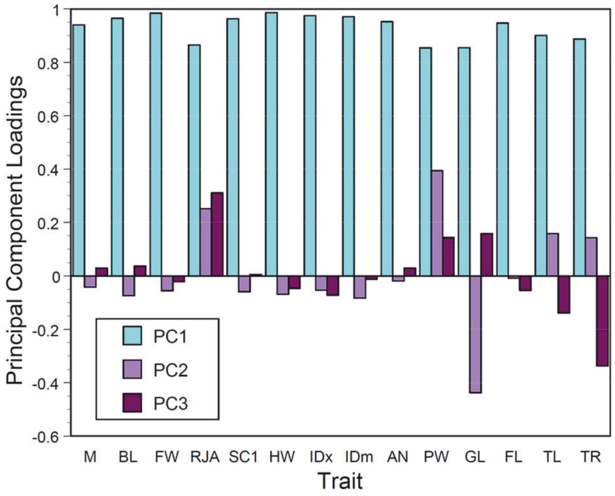

Fig. 2 Principle component analysis reveals new morphological variables. Principal component loadings for PC1, 2, and 3, are represented by light blue, light purple, and dark purple bars, respectively. Traits are abbreviated as M mass, BL body length, FW forewing length, RJA reslin joint area length, SC1 submarginal cell 1 length, HW head width, IDx maximum interorbital distance, IDm minimum interorbital distance, AN antenna length, PW pronotum width, GL second segment gaster length, FL femur length, TL tibia length, and TR tarsus length.

relationship between castes (i.e., similar slopes and different intercepts) (Fig. 3). In other words, traits got larger as body size got larger in both castes, but in different proportions regardless of mass (Shingleton et al. 2007). Thus, the majority of queen traits were proportionately larger than worker traits. Notably, the correlational strength of the mass-trait relationship varied widely from somewhat strong (e.g., queen body length) to weak (e.g., tibia length, tarsus length), with most traits showing relatively weak correlations. Interestingly, there were two traits (body length and gaster length) that had significantly different slopes between castes, indicating that the size of these traits scaled differently with body mass in the two castes (Fig. 3, Supplementary Table S3).

Genetic effects on morphological traits

We investigated if variation in morphological traits was influenced by genetic variation by determining if daughters derived from different patrilines differed significantly in trait size. All colonies used in this study had queens that mated with 5–8 different males, which is typical for this species (Orr et al. 2024). We found significant patriline effects on two queen traits (reslin joint area length and PC3) and two worker traits (reslin joint area length and submarginal cell 1 length) using the Wald test on our nested random effects model (Queen Patriline Effects and Worker Patriline Effects in Table 1).

We also calculated narrow-sense heritability (h^2) for all traits. Our estimates of heritability were modest and did not exceed 0.3 for any trait. We compared the heritability of traits between castes and found that queen traits were significantly more heritable than worker traits ($t = 5.054$, df = 13, $P = 0.0002$) (Fig. 4, Supplementary Table S4). Next, we explored the relationship of heritability estimates between castes for all traits. That is, we aimed to determine if traits that showed high (low) heritability in queens also showed high (low) heritability in workers. We found a significant relationship between trait heritability in queens and workers ($r = 0.6984$, $P = 0.0055$) (Fig. 4, Supplementary Table S4).

Further, we explored the effect of colony on morphological trait variation among both queen and worker castes. We found that queens and workers from different colonies differed significantly in trait size for many traits (Queen Colony Effects and Worker Colony Effects in Table 1). Overall, these results indicate strong environmental, and modest genetic, effects for most traits in both castes (Table 1).

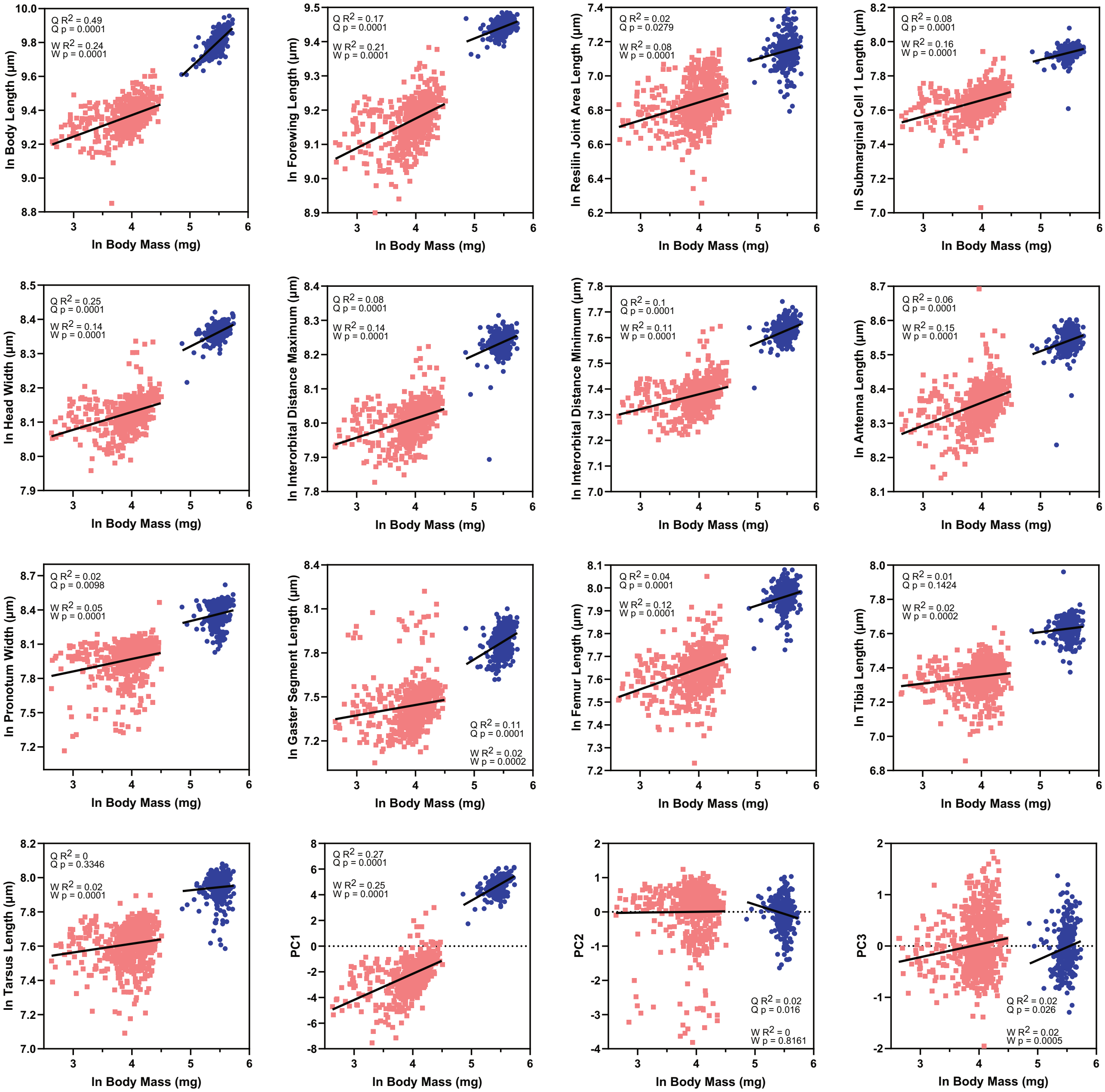

Fig. 3 Scaling relationships in queen and worker *V. maculifrons* castes. Most traits scale strongly and positively with mass and most scaling relationships display similar slopes in queens and workers. The red squares represent workers, and the blue circles represent queens. Queen (Q) and worker (W) R^2 and *p*-values are listed on each corresponding plot.

Correlations between queen and worker castes

Caste correlations may exist for traits that have significant genetic effects in both castes. Indeed, we found that the strongest within colony caste correlation (WCCC) of 0.514 was associated with the reslin joint area length trait that had significant genetic effects in both castes (Table 1, Supplementary Fig. S3, Supplementary Table S5). This was the only trait that displayed a statistically significant WCCC ($Z = -2.355$, $P = 0.0185$). No other trait in our study showed significant genetic effects in both castes and, therefore, WCCCs were modest and not significant for other traits (Table 1, Supplementary Table S5, Supplementary Fig. S3).

We also examined the relationship between queen and worker castes when data from all colonies and patrilines were combined (across colony caste correlation; ACCC, Fig. 5, Supplementary Fig. S3, Supplementary Table S5). Generally, we found a strong, significant, linear relationship between castes for most traits (Table 1). This indicates that colonies that produced large (small) queens also tended to produce large (small) workers.

We next investigated if there was an association between caste dimorphism and WCCC or ACCC for each trait (Fig. 6). A negative relationship between these variables is expected if traits with weak caste correlations are able to freely evolve to their optimal dimorphic values (Cox and Calsbeek 2009; Wyman et al. 2013). However, we found a nonsignificant relationship between WCCC and trait dimorphism ($F = 0.14$, $P = 0.7122$, Fig. 6). Similarly, we examined the relationship between ACCC and trait dimorphism, which also could provide evidence of conflict within the genome and inform about caste antagonistic variation. However, we also found a nonsignificant relationship between ACCC and trait dimorphism ($F = 2.55$, $P = 0.1414$, Fig. 6).

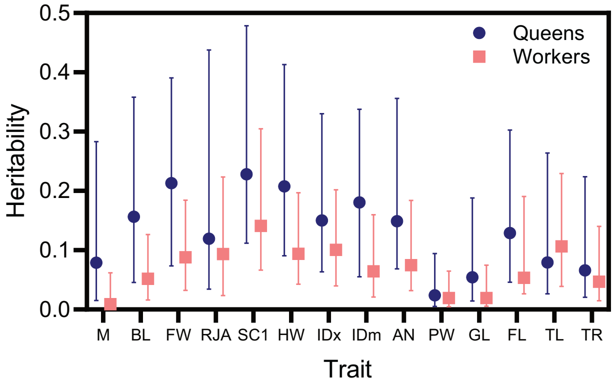

Fig. 4 Heritability for 14 morphological traits in queen and worker *V. maculifrons*. Heritability estimates for traits were significantly higher in queens than workers, and heritability was correlated in the two castes. Traits are abbreviated as M mass, BL body length, FW forewing length, RJA reslin joint area length, SC1 submarginal cell 1 length, HW head width, IDx maximum interorbital distance, IDm minimum interorbital distance, AN antenna length, PW pronotum width, GL second segment gaster length, FL femur length, TL tibia length, and TR tarsus length. The red squares represent workers, and the blue circles represent queens.

DISCUSSION

Individuals within species display remarkable phenotypic variation. Such phenotypic variation arises because individuals experience different environments as they develop and because individuals possess genetic differences that affect phenotype. The goal of this study was to quantify the genetic and environmental effects on the dimorphic queen and worker castes in a social wasp. Our primary interest was to investigate the variation in caste size and shape in order to understand selective pressures operating on caste evolution. We hoped that by studying the effects of genotype on traits in social insect castes, we could gain greater insight into the evolution of phenotype in social systems (Jeanson and Weidenmüller 2014).

Shape and size variation within and between castes

Queen and worker castes differ in size. We found that the morphology of queen and worker castes varied considerably; the means of queen traits were always larger than the means of worker traits. Such morphological variation is a defining feature of highly social insects (Trible and Kronauer 2017; Treanore et al. 2021). In addition, our data showed a much larger variance among all measured traits in workers than queens. This difference in size variation between castes may have functional consequences for the individual queens and workers.

Workers show high variance in traits in many social insect taxa (Toth et al. 2016; Kelemen et al. 2022). However, the high variability in size in workers has not been noted before in the social wasps. Previous morphological work in *V. maculifrons* found only two measured traits (thorax width and length) to have significantly more variation in workers than queens (Kovacs et al. 2010a, 2010b). Additional research in paper wasps (*Polistes* sp.) found no significant differences in size variance between workers and queens (Montagna and Antonialli-Junior 2016; Miller and Sheehan 2021). This may be in part due to the weak caste dimorphism associated with paper wasps that have less distinguishable castes and simpler societies than the *Vespula* wasps studied in this investigation (Yoshimura and Yamada 2018).

High worker size diversity may be important to insect societies because workers of different sizes may undertake different tasks (Oster and Wilson 1978). In contrast, variation in queen size is not generally thought to be adaptive for individual queens in social insect species that found colonies independently. Natural selection should select for large queens because many aspects of queen success, such as robust flight, colony founding, and individual reproduction, rely on large body size (Kovacs et al. 2008; Kovacs and Goodisman 2012; Kelemen et al. 2022). Indeed, previous research has found a strong correlation between body size and fitness in social insect reproductives (i.e., bigger is better, particularly for independent-colony-founding queens) (Kingsolver and Pfennig 2004; Kovacs and Goodisman 2012; Wright et al. 2019). Overall, the difference in variance among *V. maculifrons* castes confirms that importance of their respective functions in colonies.

Lack of Strong Caste Dimorphism. Selection has led to substantial queen-worker dimorphism in many species of hymenopteran social insects (Wilson 1953; Fairbairn 1997; Smith 2023). Moreover, queen-worker dimorphism is a strong indicator of social function in social insects (Beekman and Oldroyd 2019; Ohyama et al. 2023). We found that *V. maculifrons* displayed little variation in caste dimorphism among traits. Most of the measured traits had dimorphism values between 0.1 and 0.2. Our findings are similar to other social wasp studies (Kovacs et al. 2010; Perrard et al. 2012). In general, caste dimorphism is moderate in social wasps and bees (Leimar et al. 2012) and more extreme in ants and termites (Noirot 1989; Wheeler 1991; Roisin 2000).

Caste dimorphism represents a special case of phenotypic dimorphism, which occurs in many species in some form. The most widespread form of phenotypic dimorphism is sexual dimorphism which, like caste dimorphism, primarily arises from differential gene expression from a common genome (Hopkins and Kopp 2021). Most insect species have larger bodied females compared to males, which can be attributed to additional female instars. This female-biased sexual dimorphism is typically moderate across taxa (Teder et al. 2021), but can be large in extreme cases such as the spongy moth *Lymantria dispar* or parasitoid wasp *Sclerodermus harmandi*. Therefore, it is possible that selection pressure would apply more strongly on the queen caste in *V. maculifrons*. However, this is unlikely given the small to moderate dimorphism values of the traits measured in this study.

Queen and worker castes differ in shape. We investigated how the shape of queen and worker *V. maculifrons* traits varied as a function of size, and whether the relationships differed between castes. Allometric relationships between trait size and mass revealed interesting scaling relationships in *V. maculifrons* queens and workers. Analysis for most traits revealed positive, linear correlations. In other words, traits got larger as the individual got larger in a roughly linear fashion, which is a widely known phenomena (Brown and West 2000).

In contrast, PC2 was significantly negatively correlated with body mass in queens. PC2 was highly influenced by negative loadings on gaster length and positive loadings on pronotum width, reslin joint area length, tarsus length, and tibia length (Fig. 2). We thus hypothesized that PC2 may represent a 'body thickness' characteristic that incorporates both body length and body width measurements. Interestingly, a previous study observed that more slender *V. maculifrons* queens had improved success in overwintering (Kovacs and Goodisman 2012), suggesting that body thickness may be under selection.

Our results also revealed that the relationships between trait size and body size for almost all traits, except body length and gaster length, were similar between castes (i.e., lines had similar slopes, Fig. 3); however, the y-intercept for the allometric line in queens was usually larger than the y-intercept for workers. This particular allometric relationship indicates that queens had larger proportional trait sizes than workers, regardless of mass (Nijhout and Wheeler 1996; Fairbairn 1997; Shingleton et al. 2007). Similarly, previous research has uncovered that *Vespa crabro* and *Vespa velutina* queens and workers displayed similar wing

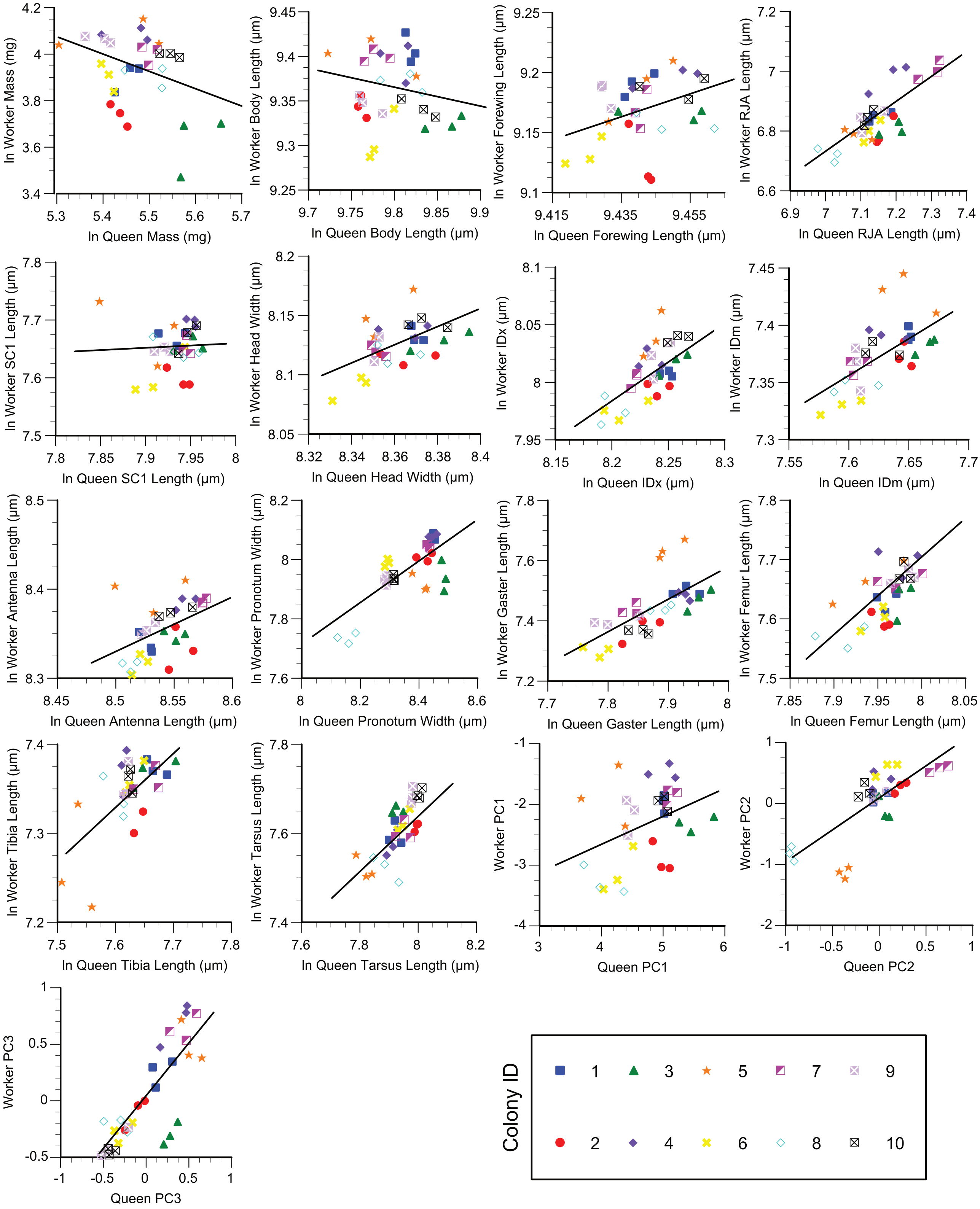

Fig. 5 The across colony caste correlation for *V. maculifrons* traits. A positive relationship between worker size and queen size is evident for most traits. Each data point represents a patriline and each distinct symbol indicates membership in a different colony.

allometry (i.e., slopes) with different lateral transposition (i.e., intercepts) (Perrard et al. 2012). Thus, it is clear that queen and worker castes in *V. maculifrons* differ in both size and shape (McCoy et al. 2006; Perrard et al. 2012), albeit less dramatically than other social insect taxa, such as ants and termites.

Most of the allometric slopes examined in this study had modest, positive slopes, suggesting that morphological traits scaled with body mass. However, we observed the steepest slopes

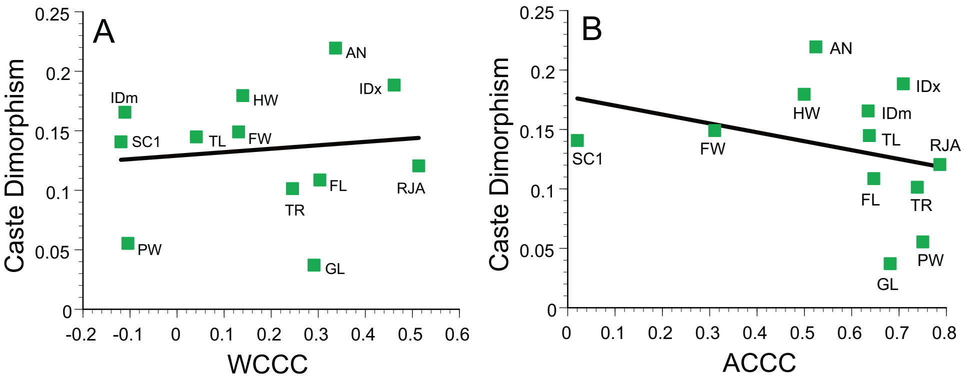

Fig. 6 Weak relationships between caste correlations and caste dimorphism reveal a lack of evolutionary conflict. The relationship between caste dimorphism and (**A**) mean 'within colony caste correlation' (WCCC) values and (**B**) 'across colony caste correlation' (ACCC) for all traits in *V. maculifrons*. Traits are abbreviated as M mass, BL body length, FW forewing length, SC1 submarginal cell 1 length, RJA reslin joint area length, HW head width, IDx maximum interorbital distance, IDm minimum interorbital distance, AN antenna length, PW pronotum width, GL second segment gaster length, FL femur length, TL tibia length, and TR tarsus length.

for queen body length, gaster length, and pronotum width. The consistent finding of caste proportional differences among most traits was one of the most unexpected findings in this study. That is, queens tended to show larger traits for their body size than workers. Allometric scaling has been considered a possible constraint in phenotypic evolution (Pélabon et al. 2014), which may also play a role in social insect caste evolution. Future studies comparing the allometric scaling of all three castes (queens, workers, and males) across multiple social wasp species could reveal additional important insights.

Modest heritability of caste phenotypes

Variation in only a few traits was significantly influenced by genetic variation in workers (reslin joint area length and submarginal cell 1 length) and queens (reslin joint area length and PC3). The relatively low heritability values and lack of genetic effects on trait size indicate that *V. maculifrons* queens and workers may possess relatively limited genetic variation influencing the variability of morphological traits. Thus, directional selection would be ineffective in the two castes because they apparently lack substantial genetic variation influencing trait size. It is possible that stabilizing selection may explain the lack of genetic variation found in our study. It may also be the case that selection for optimal trait size in *V. maculifrons* might have eliminated standing genetic variation for those traits, thus reducing heritability. Alternatively, our results may reflect particularly strong environmental variation influencing trait variation.

Previous studies have reported varying estimates of heritability in social insects. For example, a lack of heritable morphological variation via patrilines was found in the worker caste of the ant, *Eciton burchelli* (Winston et al. 2017). The researchers suggested that most of the morphological variation arose from either environmental conditions or maternal genotype, rather than patriline genotype. Other studies have uncovered weak heritability estimates for morphological variation in social insects and championed a stronger environmental influence instead (Bargum et al. 2004; Skaldina and Sorvari 2020).

There is evidence that variation in morphology may be more strongly influenced by genetic variation than other phenotypes (e.g., behavioral phenotypes or susceptibility to disease) (Wills et al. 2018). Recent work has found moderately high heritability estimates for morphological traits compared to behavioral or physiological traits in social bees (Koffler et al. 2017) and ants (Walsh et al. 2020). However, some research has suggested that behavioral phenotypes are also strongly influenced by genetic variation (Constant et al. 2012). Therefore, social insect taxa may display a wide range of heritability values for various phenotypic traits.

Interestingly, variation in one trait, reslin joint area length (RJA), was influenced by genotype in both castes in our study of *V. maculifrons*. Previous research has found that the resilin joint area is important in wing flexibility, flight stability, load-lifting capacity, and vertical force production in wasps (Mountcastle and Combes 2013, 2014). Resilin, an elastomeric protein found in many arthropods, is one of the most elastic proteins known in nature (Elvin et al. 2005). It is unclear if the length or size of this joint area could have implications in flight, but we speculate that wasps with longer RJAs may have improved flight capabilities that would increase foraging efficiency, mating success, and colony establishment. Future research should investigate the fitness and evolutionary consequences associated with different wing morphologies and test if worker and queen trait optima are different.

One of the principal components we analyzed, PC3, showed particularly interesting associations with genotype. Variation in PC3 was significantly influenced by genotype in *V. maculifrons* queens. PC3 displayed positive loadings on gaster length and reslin joint area length and negative loadings for tarsus length and tibia length (Fig. 2). These loadings suggest genetic effects of relative leg length or shape in queens. A recent morphological study in *Camponotini* ants also identified a principal component as an indicator of leg length (Laciny et al. 2019). Longer legs may evolve for a variety of reasons among insect taxa including thermoregulation (Sommer and Wehner 2012), jumping ability (Burrows and Sutton 2008), mating success (Amin et al. 2012), and food acquisition (Pauw et al. 2017). However, little is known about the importance of leg length in social wasps. We speculate, along with others (Perez and Aron 2020), that longer legs may affect wasp fitness.

Remarkably, almost all morphological traits we measured were more heritable in queens than workers (see also Bargum et al. 2004). This indicates that genetic variation has stronger influences on trait variation in queen than worker *V. maculifrons* wasps. It is possible that workers have been subject to greater selection on morphology thereby leading to a loss of associated genetic variation in the focal traits. However, selection is typically expected to operate less strongly on worker traits than queen traits because worker traits are generally subject to indirect selection whereas queen traits are subject to direct selection (Linksvayer and Wade 2009; Hall and Goodisman 2012). In principle, genes associated with queen-expressed traits should, therefore, be less genetically variable than genes associated with worker-expressed traits if directional selection is the predominate mode of selection operating in a system. And one may predict that heritability would consequently be lower in queens than workers. Therefore, it is surprising that heritability measurements in *V. maculifrons* queens were higher than in workers queens.

We found a significant relationship between heritability estimates of queens and workers. That is, traits that were highly heritable in queens also tended to be highly heritable in workers. This result suggests that there is a similar, albeit modest, genetic basis to morphological variation in both queens and workers. Thus, similar genetic architecture may influence trait size in both castes. This modestly significant correlation between queen and worker heritability values in *V. maculifrons* might lead to the opportunity for some types of genetic conflict. That is, this result suggests some level of genetic similarity in the way that variability in queen and worker traits develop. Thus, it is possible that selection on trait size operating in one caste could affect phenotype in the other caste (Pennell and Morrow 2013).

Lack of evidence for genetic conflict

Distinct phenotypic classes may not be able to reach their optimal phenotypes because of genetic conflict (Yang and Andrew Pospisilik 2019; Yoon et al. 2023). To test for the presence of resolved genetic conflict, we examined the relationship between the Within or Across Colony Caste Correlation (WCCC or ACCC) and dimorphism values for our measured traits in *V. maculifrons*. Theory predicts a negative relationship between dimorphism and caste correlation if traits evolved under the pressure of genetic conflict; a negative association indicates that traits that are weakly genetically correlated between castes are able to evolve into their opposing dimorphic optimums (Cox and Calsbeek 2009). In contrast, traits that that show strong caste correlations will be unable to evolve optimal phenotypes in the two castes and therefore show low dimorphism values.

We examined the relationship between dimorphism values and the Within Colony Caste Correlation (WCCC) and the Across Colony Caste Correlation (ACCC). However, we found nonsignificant relationships between both WCCC and ACCC vs dimorphism. We also found many traits had WCCCs and ACCCs that were opposite in value or disparate. Thus, our data from *V. maculifrons* social wasps are not consistent with the idea that traits have evolved under genetic conflict. We note, however, that traits in queen and worker castes of *V. maculifrons* have a narrow range of relatively weak dimorphism values (0.037 – 0.219). Moreover, trait size was not significantly influenced by genotype in most cases (i.e., had low heritability). Thus, the relationships between caste correlation and dimorphism may not be fully apparent with this suite of traits analyzed in this species.

Notably, negative associations between caste or sex correlations and dimorphism have been found in many wild populations of mammals, insects, and birds (Cox and Calsbeek 2009). It remains unclear if intralocus genetic conflicts operate similarly in *V. maculifrons*. Regardless, the study of genetic conflict in social insects is worthy of further study because such conflict potentially constraints caste and social evolution.

Strong environmental effects on caste phenotypes

We found strong colony effects on most morphological traits in our study for both castes, including the principal component variables. That is, individual queens and workers from different colonies differed substantially and significantly in trait size (Table 1; Fig. 5). Additionally, we generally found a positive correlation between castes existed for both patriline and colony effects together (as measured by the Across Colony Caste Correlation, ACCC). That is, colonies that tended to produce large (small) gynes tended to produce large (small) workers.

Environmental influences on individuals from different colonies has been well documented in social insects previously (Kovacs et al. 2010a, 2010b; Smith 2023). For example, recent work in soldiers of the leaf-cutting ant found stronger environmental effects on morphology than matriline effects (Sandoval-Arango et al. 2020). The phenotypic differences between individuals from different colonies arise because such individuals differ genetically but more so because they experience different developmental environments. Our analyses showed only modest trait heritability values and limited patriline effects on variation in trait size. Notably, matriline effects may play a role in the observed trait variation, but this was not explicitly tested in this study. However, our results support the idea that environmental variation plays a stronger role in variability in trait size in this species.

It is perhaps notable that this investigation on *V. maculifrons* specifically documented environmental and genetic effects on trait size in a natural population of the species. That is, individual queens and workers analyzed in this study developed in naturally occurring, rather than laboratory-reared, colonies. Thus, it is possible that there was substantial environmental variation among colonies, leading to lower heritability estimates and lower genetic effects on trait variability overall.

PC1 had strong colony effects for both queens and workers. We posit that PC1 represents a measure of overall body size, because all traits loaded strongly and positively on it (Molet et al. 2012; Okada et al. 2013) (Fig. 2). Indeed, colony variation that arises from environmental differences has been shown to dramatically affect individual sizes of social insects (Fjerdingstad 2005; Kovacs et al. 2010a, 2010b; Wills et al. 2018; Smith 2023). There are a variety of environmental factors that could influence body size including food availability (Feinerman and Traniello 2016), nutritional quality (Dussutour and Simpson 2008; Smith and Suarez 2010), age and size of colony (Tschinkel 2010), and social form (i.e., monogyny vs polygyny) (Frumhoff and Ward 1992; Fjerdingstad and Crozier 2006). Thus, our results support the idea that variation in size is dictated by environmental effects, rather than paternal genetic variability.

CONCLUSION

Phenotypic evolution in social insects is affected by selection operating differentially on traits in distinct castes. We show evidence for patriline effects for a few morphological traits in a social wasp and a relationship between heritability values between queens and workers. However, our findings suggest that phenotypic variation within castes in this species is determined largely by environmental variation. Our study also robustly uncovered interesting allometric relationships between castes. Thus, overall, this study emphasizes the importance of the relationship between genetic and environmental variability in determining variation in individual morphology. We suggest that future investigations into the genetic architecture governing the development of distinct phenotypes within species will be helpful in understanding the evolution of phenotypic diversity.

DATA AVAILABILITY

Data has been deposited with DRYAD: https://datadryad.org/stash/share/-6vbC_EUConsGh4vWYs_oJBdgU19vizbsaXB087aFa4.

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ACKNOWLEDGEMENTS

Research funding provided by NSF-DEB: 2105033 and NSF-IOS: 2019799 to M.A.D.G. and USDA: 2023-67012-39886 to S.E.O. We would like to thank Paige Caine and Andrew Robertson for their assistance with collecting the wasp colonies.

AUTHOR CONTRIBUTIONS

SEO and MADG conceived the work and designed the research; SEO, NAH, KAM, AKP performed the research and collected the data; SEO and JLK analyzed the data with support from MADG; SEO wrote the manuscript with support from MADG.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL

This study was conducted using *Vespula maculifrons*, an unregulated invertebrate species. Therefore, no ethical committee approval was required.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41437-024-00701-5.

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