



Detecting selection on morphological traits in social insect castes: the case of the social wasp *Vespula maculifrons*

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Highly social insects dominate terrestrial ecosystems because society members belong to discrete castes that undertake distinct tasks. The distinct functional roles of members of different castes may lead to divergent selective regimes, which may ultimately lead to morphological specialization and differentiation of the castes. This study used morphological and genetic analyses to identify traits that experienced caste-specific selection in the social wasp *Vespula maculifrons* (Buysson, 1905). Traits putatively under selection were identified based on their degree of caste dimorphism, levels of variability, strength of correlations with other traits, and patterns of allometric scaling. Analyses of trait characteristics suggested that queen thorax length, thorax width, and possibly mass, have experienced queen-specific selection. Additionally, trait dimorphism and intercaste phenotypic correlation values were negatively correlated, as expected if some morphological traits were subject to selection, leading to alternate phenotypic optima in the two castes. Overall, our analyses demonstrate how techniques used to identify selection between dimorphic groups can be applied to social species with distinct castes. In addition, our analyses suggest the operation of selection may be stronger in reproductive than in non-reproductive castes. © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, **101**, 93–102.

ADDITIONAL KEYWORDS: allometry – dimorphism – genetic architecture – genetic correlation – Hymenoptera – morphology – social insect – yellowjacket.

INTRODUCTION

Individual members of most animal species belong to distinct classes, which perform different functional roles (e.g. the sexes; Andersson, 1994; Chippindale, Gibson & Rice, 2001). The distinct roles undertaken by members of different classes may lead to class-specific selective regimes, which can differentially affect the evolution of morphological traits within classes. Ultimately, homologous traits in each class may evolve towards separate phenotypic optima, which can lead to dimorphism among individuals in

the population (e.g. sexual dimorphism; Lande, 1980; Fairbairn, 1997; Reeve & Fairbairn, 2001).

Distinct classes have evolved in many social insect species (ants, termites, some bees, and some wasps; O'Donnell, 1998; Noll, Wenzel & Zucchi, 2004). For instance, hymenopteran social insect females are divided into queen and worker castes (Wilson, 1971). Generally, queens are the primary reproductive females, and are responsible for founding new colonies. In contrast, workers forage, maintain the nest, and care for the brood. Many social insect queens show dramatic morphological differences from conspecific workers. Moreover, queen–worker caste differences generally arise via environmental determination, thus making the caste system a key example of polyphenism, whereby the same genotype generates distinct phenotypes (Wheeler, 1986; Goodisman, Kovacs & Hunt, 2008).

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Queens and workers in ancestral social insect populations probably responded to, and in turn generated, divergent selective pressures, which ultimately resulted in morphological differentiation between the castes. The purpose of this study was to identify the past operation of selection on caste dimorphic traits using classic morphological analyses that have not previously been applied to caste systems. Specifically, we aimed to discern the effects of caste-specific selection in a strongly caste-dimorphic social wasp by incorporating methods typically used to identify the action of sexual selection on morphological traits.

We first attempted to identify the operation of selection in social insect castes by analysing individual morphological traits in four different ways. First, we calculated the level of trait dimorphism between castes. Caste-specific selection may move traits within castes to different phenotypic optima, as sometimes occurs for traits under sex-specific selection (Lande, 1980; Reeve & Fairbairn, 1996, 2001; Bonduriansky & Rowe, 2005). Consequently, divergent selection between castes would lead to strong dimorphism for particular traits over time. Thus strong trait dimorphism in contemporary social insect populations would provide possible evidence of past selection within one or both castes.

Second, we compared levels of trait variation between castes. A trait experiencing strong stabilizing or directional selection in one caste is expected to display lower levels of phenotypic variation than the same trait in the other caste, provided that genetic variation influences phenotypic variation in similar ways in both castes (Falconer & MacKay, 1996; Kovacs *et al.*, 2010). Thus, significant differences in the level of trait size variation between castes may reflect the past effects of selection.

Third, we determined whether morphological traits were correlated within castes (i.e. intracaste correlations). Intracaste trait correlations arise when a change in size of one trait leads to a correlated change in size of another trait. Such correlations may constrain phenotypic evolution, because a particular trait may be unable to evolve independently of another. However, strong antagonistic selective pressures may ultimately result in a reduction of intracaste correlations (Cheverud, 1984; Price & Langen, 1992). As a result, traits under high historical levels of caste-specific selection are expected to be less correlated with other morphological traits than traits not under historically strong selection.

Fourth, we compared allometric relationships between castes (Wilson, 1953, 1971; Wheeler, 1991; Nijhout & Wheeler, 1996; O'Donnell, 1998; Tschinkel, Mikheyev & Storz, 2003; Bonduriansky, 2007b). Caste-specific allometric patterns are often taken as evidence of functional differences between queen and

worker castes (Hunt *et al.*, 1996), or among worker subcastes (Wilson, 1953; Oster & Wilson, 1978; Diniz *et al.*, 1994; Fraser *et al.*, 2000; Schoning, Kinuthia & Franks, 2005). Thus significant differences in trait allometry between the queen and worker castes might reflect past differential selective pressures operating in the two castes. In summary, we analysed four different aspects of *individual* morphological traits (i.e. dimorphism, variability, intracaste correlation, and allometry) to identify the action of past caste-specific selection.

In addition, we assessed relationships of *all* morphological traits analysed to determine if the suite of traits as a whole displayed evidence of having been acted on by selection. Specifically, we determined the association between intercaste correlations (cf. intracaste correlations above) and caste dimorphism values. The importance of this relationship arises from the fact that homologous traits in castes of ancestral social species were probably controlled by the same genetic mechanisms. This common genetic control of traits in different castes would have resulted in high intercaste genetic correlations (i.e. a particular genotype would have the same phenotypic effect in both castes; Lande, 1980; Bonduriansky & Rowe, 2005). However, highly correlated traits, which experienced divergent selective regimes between castes in ancestral populations, would have been suboptimally expressed in both castes (Chippindale *et al.*, 2001; Bonduriansky & Rowe, 2005; Bonduriansky, 2007a). Selection would thus have acted to remove such antagonistic constraints on fitness by reducing intercaste genetic correlations, thereby allowing dimorphic phenotypes to evolve more rapidly (Lande, 1980; Reeve & Fairbairn, 1996, 2001; Bonduriansky & Rowe, 2005; Fairbairn & Roff, 2006; Bonduriansky & Chenoweth, 2009). Consequently, traits under strong selection in one or both castes are expected to show high levels of trait dimorphism (see above) and low intercaste correlations. In contrast, traits not under strong differential selection in the castes may show low levels of trait dimorphism and high intercaste correlations. Thus, overall, a negative association between intercaste phenotypic correlation and trait dimorphism may be expected if the traits being analysed were historically genetically correlated, and differed in the levels of caste-specific selection that they had experienced.

This study attempted to identify the effects of caste-specific selection in the eastern yellowjacket *Vespula maculifrons* (Buysson, 1905). This highly social wasp displays a high degree of differentiation between its queen and worker castes (Fig. 1; Spradbery, 1973; Greene, 1991). Moreover, *V. maculifrons* castes are expected to be under distinct types of selection because of their strongly divergent morphologies, behaviours, reproductive roles, and functions (V.

maculifrons natural history reviewed by Spradbery, 1973; Macdonald & Matthews, 1981; Greene, 1991; Hoffman & Goodisman, 2007). The social structure displayed by *V. maculifrons* is also well understood: colonies are headed by a single, multiply-mated queen (Ross, 1985; Goodisman, Kovacs & Hoffman, 2007a), and members of all patrines are found in both castes (Goodisman, Kovacs & Hoffman, 2007b). Thus the social system and variation in caste functions displayed by *V. maculifrons* facilitates an analysis of the effects of caste-specific selection. Overall, we expected that queen traits would show stronger evidence of being under historical levels of caste-specific selection than worker traits, because queens may be more directly subject to the effects of both natural and sexual selection. In contrast, workers may experience selection more indirectly through their effects on colony success, and thus may not display the effects of selection as strongly (Linksvayer & Wade, 2009).

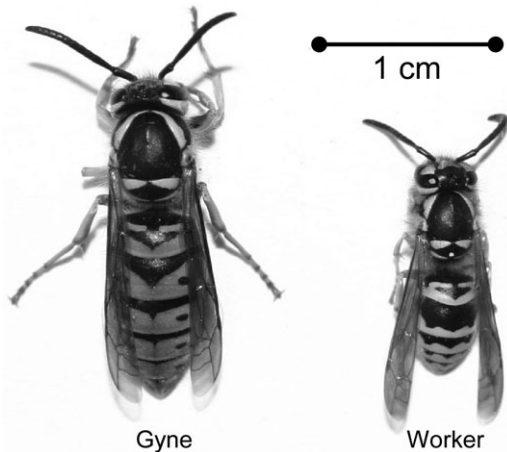


Figure 1. *Vespsula maculifrons* gyne (pre-reproductive queen) and worker.

MATERIAL AND METHODS

SAMPLES

We collected 18 *V. maculifrons* nests from metropolitan Atlanta, Georgia, USA, in 2004 and 2005. Six colonies contained only workers, 11 colonies collected in the autumn contained both gynes (pre-reproductive queens) and workers, and only gynes were collected from one additional colony. A mean ($\bar{x} \pm \text{SD}$) of 49.33 ± 35.02 workers and 37.58 ± 6.95 gynes per colony were sampled (Table 1). No obviously general individuals were included in the analyses.

GENETIC ANALYSIS

We assigned gynes and workers to patrines using microsatellite markers. DNA was extracted from a single leg of each individual worker or gyne so as not to substantially affect the size measurements taken. Worker genotypes were then determined at eight microsatellite loci: LIST2003, LIST2004, LIST2013, LIST2019, LIST2020, RUFA 5, VMA-3, and VMA-6. The heterozygosities at these loci were sufficiently high so that the probability of two males from the population having the same genotype was low ($\ll 0.0001$; Goodisman *et al.*, 2007b), and thus individual workers could readily be assigned to patrines (Johnson *et al.*, 2009). Gyne genotypes were determined at a subset of loci (LIST2004, LIST2013, LIST2019, LIST2020, and VMA-6), which were variable enough to distinguish all patrines. Polymerase chain reactions (PCRs) were conducted as described by Hoffman, Kovacs & Goodisman (2008), and were visualized using an Applied Biosystems PRISM@ 3100 Genetic Analyzer.

MORPHOLOGICAL MEASUREMENTS

Workers and gynes were dried overnight at 60°C in order to obtain their dry mass. Each individual was

Table 1. Sample sizes (n), means, standard deviations (SDs), and coefficients of variation (CVs) for five traits in *Vespsula maculifrons* gynes and workers

Trait	Workers			Gynes			Between castes t
	n	Mean \pm SD	CV	n	Mean \pm SD	CV	
Thorax width	860	2.31 ± 0.24	0.10	448	3.61 ± 0.18	0.05	-8.90***
Thorax length	862	3.65 ± 0.37	0.10	447	5.49 ± 0.33	0.06	-4.66***
Third tergum length	859	1.66 ± 0.22	0.11	448	2.82 ± 0.35	0.12	0.41
Gaster length	843	5.65 ± 0.77	0.14	424	9.72 ± 1.41	0.14	-0.34
Mass	883	14.68 ± 3.16	0.22	450	88.09 ± 24.68	0.28	2.40**

The between caste Student's t -test values result from tests comparing worker and gyne CVs. Significant t -values indicate that CVs differed between castes. Negative, or positive, Student's t -test values indicate that the CV of gynes was less than, or greater than, that of workers, respectively.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

then digitally photographed in a dorsally facing position. A Zeiss millimetre micrometer was included in each photo for calibration. Using tools within Canvas v9.0.4, we measured the linear dimensions of the following five traits: thorax width (measured from the inside of the left wing tegula to the inside of the right wing tegula, i.e. the width of the mesoscutum), thorax length (measured from the anterior tip of the mesoscutum to the posterior tip of the propodeum, i.e. the mesosoma), length of the third tergum, gaster length (the measure of the abdomen, not including the first abdominal tergum), and total body length. Body length was used as the indicator of body size for caste dimorphism estimates (see below) and bivariate allometric estimates (Fig. 2).

Contemporary analyses of social insects suggest that some of the analysed traits may be of functional importance. For example, mass and body length are related to survival and fecundity in a variety of social insects (Heinze, 1989; Elmes, 1991; Ruppel, Heinze & Hölldobler, 1998; Abell *et al.*, 1999; DeHeer, 2002; Wiernasz & Cole, 2003; Bargum, Boomsma & Sundström, 2004; Fjerdingsstad, 2005). Additionally, gaster size often exhibits pronounced allometric caste differences (Blackith, 1958; Jeanne & Fagen, 1974; Turillazzi *et al.*, 1994). Recent studies also suggest that gaster length and body length of *V. maculifrons* gynes are correlated with mating success, as well as overwintering survival (Kovacs, Hoffman & Goodisman, 2008; Kovacs and Goodisman, unpubl. data). Thus there is reason to expect that social wasp gynes experience strong selection associated with their life history (Molina, Harris & O'Donnell, 2009).

STATISTICAL ANALYSES

We determined the degree of trait dimorphism between workers and gynes using a modification of Bonduriansky & Rowe's (2005) formula, originally derived to calculate the trait dimorphism between males and females. The degree of caste dimorphism for each trait i was thus calculated for each colony separately as $D_i = 1 - (\bar{X}_i^W / \bar{X}_{BL}^W) / (\bar{X}_i^G / \bar{X}_{BL}^G)$; where \bar{X}_i^j is the mean absolute size of trait i in caste j (W denotes worker and G denotes gyne), and \bar{X}_{BL}^j is the mean total body length of caste j . The D_i estimate for each trait within each colony was then averaged

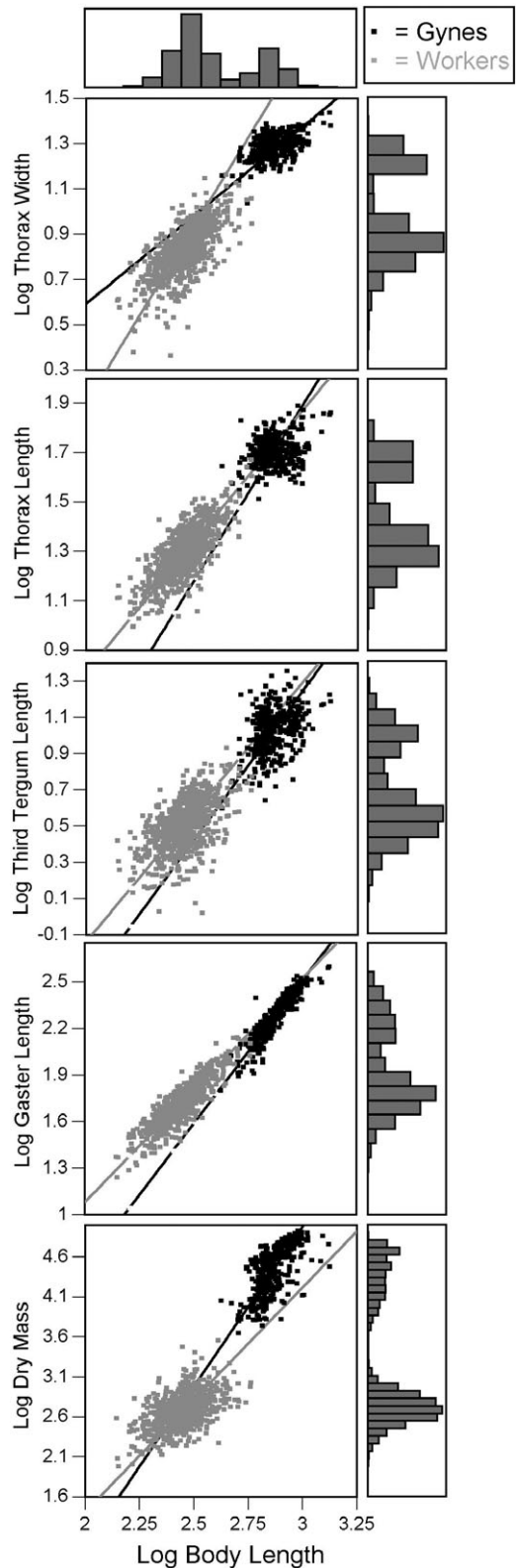


Figure 2. Relationships between log-transformed morphological traits and total body size in *Vespula maculifrons* gynes and workers. Standard major axis regression lines demonstrate a linear fit. Histograms illustrate the distributions of trait sizes in both castes. Gyne traits are represented by black symbols and lines, whereas worker traits are represented by grey symbols and lines.

across colonies to provide an overall point estimate of trait dimorphism. This calculation provided an estimate of relative dimorphism between traits unbiased by differences in the number of individuals sampled per colony (Bonduriansky & Rowe, 2005). Finally, Student's *t*-tests were used to determine whether the colony-level D_i estimates for each trait differed significantly from a mean of zero.

We tested if workers and gynes differed in trait variability. Specifically, we calculated the coefficient of variation (*CV*) for each trait within each caste of the eleven colonies that contained both workers and gynes (Fjerdingstad & Crozier, 2006; Roux, Roux & Korb, 2009); these colony values were then averaged to provide a point estimate for each trait's *CV* (i.e. colony information was weighted equally). Individual gyne and worker *CV*s for each colony were then used in paired Student's *t*-tests to determine if the *CV*s of particular traits differed significantly between castes.

We calculated Spearman's correlations between traits for each caste within each colony (i.e. intracaste correlations). These colony-level correlations for each trait were then averaged across colonies to provide an overall point estimate of trait correlations. Intracaste phenotypic correlations were used as proxies for genetic correlations, because phenotypic correlations generally follow genetic correlations closely (Cheverud, 1988; Bonduriansky & Rowe, 2005). The independent Spearman's correlations calculated for each colony were then used in Student's *t*-tests to determine whether the gyne or worker correlations were significantly different from zero. Additionally, the colony-level correlations for each caste were compared against each other using Student's *t*-tests to determine whether trait pairs were significantly more correlated in one caste than the other.

Allometric relationships were measured using the multivariate coefficient k , both within and among castes. k is an index of the growth 'curve' of one trait in relation to another (Strauss, 1987). Generally, a linear trait is isometric when $k = 1.0$, is positively allometric when $k > 1.0$, and is negatively allometric when $k < 1.0$. The k values for each caste were calculated for individuals within single colonies using the standardized loadings of the first eigenvector on all traits resulting from principal component analyses of the covariance matrix (Jolicoeur, 1963; Humphries *et al.*, 1981; Diniz *et al.*, 1994). Means and standard errors for k were obtained by averaging the independent k estimates obtained for each colony. We tested if the colony-level estimates of k differed significantly between the castes by using Student's *t*-tests (Diniz *et al.*, 1994). We then determined whether the colony-level estimates of k differed from the point estimates expected under isometry for each caste (Diniz *et al.*, 1994; Bonduriansky, 2007a). For all linear traits,

isometry would result in $k = 1.0$. However, mass is expected to increase geometrically with other linear measurements of size, because volume is a product of three dimensions. Thus, an isometric relationship between mass and overall body size would result in $k = 3.0$ (West, Brown & Enquist, 1997).

Intercaste correlations were obtained from combined analyses of morphological and genetic data. For each trait, Spearman's rank correlations were used to test for phenotypic correlations between full-sibling queens and workers within each colony. The overall intercaste mean phenotypic correlation and the standard error for each trait were then calculated from the individual colony estimates. Additionally, the colony-level *P* values for each independent Spearman's correlation were combined using a *Z*-transform test (Whitlock, 2005). As with the intracaste correlations discussed above, phenotypic correlations were used as proxies for genetic correlations for the intercaste correlations (Cheverud, 1988; Bonduriansky & Rowe, 2005).

RESULTS

The sizes of gyne and worker traits were almost completely non-overlapping (Fig. 2). Mass displayed the highest level of dimorphism between the two castes (Table 2). The levels of dimorphism for the other traits were considerably lower, although the values for third tergum length and gaster length were significantly greater in gynes than in workers. Moreover, worker traits tended to be smaller than gyne traits *relative* to total body length (i.e. the caste dimorphism was generally positive; Table 2).

We detected significant differences in the level of variation for a few traits between castes. Specifically, workers displayed significantly more variation in

Table 2. Mean (\pm standard error) of intercaste correlations and caste dimorphism values for *Vespula maculifrons*

Trait	Intercaste correlation \pm SE	Caste dimorphism \pm SE
Thorax width	0.222 \pm 0.159	0.028 \pm 0.013
Thorax length	0.163 \pm 0.122	-0.001 \pm 0.015
Third tergum length	0.097 \pm 0.144	0.099 \pm 0.012
Gaster length	0.182 \pm 0.141	0.134 \pm 0.012
Mass	-0.068 \pm 0.107	0.739 \pm 0.018

Negative, or positive, caste dimorphism values indicate that worker traits were larger than, or smaller than, gyne traits *relative* to the total body length, respectively. No traits displayed significant intercaste correlation. Caste dimorphism estimates that are significantly different from zero are set in bold.

Table 3. Mean Spearman's correlations between morphological traits in *Vespula maculifrons* gynes and workers

Trait	Thorax width	Thorax length	Third tergum length	Gaster length	Mass
Thorax width		0.39	0.26	0.23	0.09
Thorax length	<u>0.65</u>		0.14	0.09	0.09
Third tergum length	<u>0.42</u>	<u>0.47</u>		0.42	0.28
Gaster length	<u>0.45</u>	<u>0.45</u>	0.51		<u>0.62</u>
Mass	<u>0.40</u>	<u>0.36</u>	0.34	0.41	

Gyne correlations lie above the diagonal and worker correlations lie below. Correlations that differ significantly from zero ($P < 0.05$) are set in bold. Traits for which correlations were significantly larger in one caste than the other are underlined in the caste with the larger correlation.

Table 4. Allometric coefficients (k) for five morphological traits in *Vespula maculifrons* gynes and workers

Trait	Workers		Gynes		Castes (t)
	$k \pm SE$	Isometry (t)	$k \pm SE$	Isometry (t)	
Thorax width	0.84 ± 0.08	-2.09*	0.10 ± 0.05	-17.19***	7.83***
Thorax length	0.75 ± 0.05	-5.38***	0.05 ± 0.05	-19.99***	10.61***
Third tergum length	0.99 ± 0.09	-0.08	0.74 ± 0.12	-2.11*	1.66
Gaster length	0.96 ± 0.08	-0.48	1.15 ± 0.07	2.06*	-1.72*
Mass	1.46 ± 0.13	-11.68***	2.96 ± 0.19	-0.22	-6.41***

The t ratios result from comparing k values from expectations under isometry. Significantly negative, or positive, isometry t ratios indicate negative, or positive, allometry. The t values are also provided for tests determining whether k estimates differed significantly between castes. Negative, or positive, t ratios indicate that k estimates for gynes were greater than, or less than, those of workers.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

thorax width and length, whereas gynes showed significantly more variation in mass (Table 1). However, the coefficients of variation for the other traits did not differ significantly between the castes.

All worker trait correlations were significantly greater than zero (Table 3). Most, but not all, gyne trait correlations were also significantly greater than zero. Additionally, most trait pairs were significantly more correlated in workers than in gynes (Table 3). Thus gyne traits were not as tightly associated with each other as worker traits.

The multivariate allometric coefficients, k , were significantly different between castes for most traits (Table 4). However, the direction of allometry tended to be negative for most traits in both castes. Thus, most allometric relationships in the two castes differed in magnitude rather than direction.

Finally, we determined the relationship between caste dimorphism and mean intercaste phenotypic correlation for our five measured traits. This relationship is expected to be negative if caste-specific selection has operated on some traits (see above). As predicted, the correlation between caste dimorphism

and the intercaste correlations was significantly negative in *V. maculifrons* (Fig. 3; standard major axis regression, $y = -2.69x + 0.52$, $P = 0.02$). Thus the data are consistent with the idea that some traits have been subject to caste-specific selection and experienced a breakdown of intercaste correlation and a concomitant increase in caste dimorphism.

DISCUSSION

The goal of this study was to determine whether caste-specific selection had acted on the morphological traits of a social insect. Caste-specific selection was inferred if the trait showed: (1) substantial caste dimorphism; (2) considerable differences in variability in the two castes; (3) low intracaste phenotypic correlations; and (4) major differences in allometric relationships between castes. In addition, the association between intercaste phenotypic correlations and caste dimorphism was assessed to detect the effect of selection operating on all traits as a group. We expected that gyne traits might show more evidence of selection than worker traits, because selec-

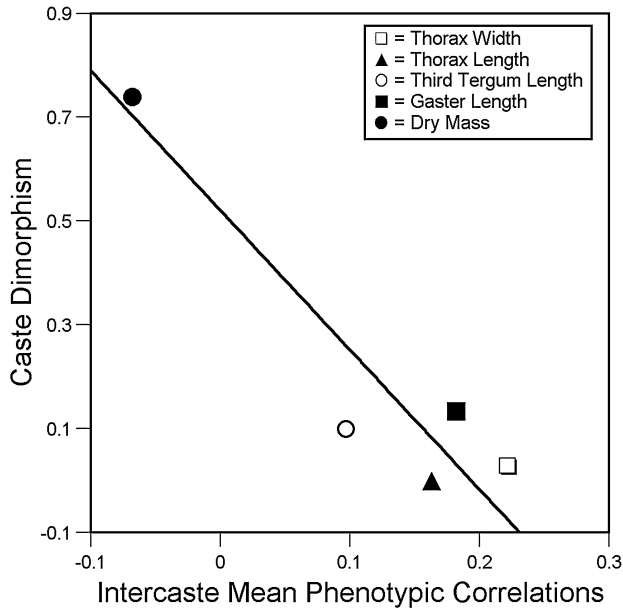


Figure 3. Relationship between levels of caste dimorphism and intercaste mean phenotypic correlations for morphological traits in *Vespula maculifrons*.

tion may have acted more directly on gynes. The combined results of our analyses suggest caste-specific selection may indeed have acted on several gyne traits, including thorax width, thorax length, and mass.

SPECIFIC TRAITS SHOWING EVIDENCE OF SELECTION

Analyses of individual traits within and between castes provided evidence of the past action of caste-specific selection. For instance, mass was highly dimorphic between the castes, whereas most other traits showed relatively low levels of dimorphism (Fig. 2; Table 2). Gyne thorax width and thorax length were also significantly less variable than homologous worker traits (Table 1). Moreover, mass and thorax width, mass and thorax length, and gaster and thorax length were not significantly correlated within gynes (Table 3). Finally, the levels of allometry differed substantially between castes for most traits. Taken together, these analyses imply that thorax width and thorax length may have been under stronger selection in gynes than in workers.

Mass also showed evidence of being under the influence of past selective pressures. Based solely on the levels of variation in mass present in the two castes, it would seem that mass was under stronger selection in workers than gynes, as gyne mass displayed significantly more variability than worker mass. However, this conclusion was not supported by gyne and worker intracaste phenotypic correlations.

Moreover, the strong correlations found between all worker traits, including mass, were not consistent with strong selection on mass in workers. It is also notable that mass is more labile in adults than other traits, and therefore may be more readily influenced by environmental factors (Mirth & Riddiford, 2007).

ALLOMETRY

Most traits displayed negative allometry (Table 4), indicating that they were relatively smaller in larger individuals than in smaller individuals. Only gyne gaster length was positively allometric. Notably, previous work in the congeners *Vespula rufa* (Linnaeus, 1758), *Vespula germanica* (Fabricius, 1793), and *Vespula vulgaris* (Linnaeus, 1758) also found that gyne gaster traits displayed positive allometry (Blackith, 1958). We also discovered that the allometric relationships differed between castes, which appears to be a common finding in social wasps (Blackith, 1958; Jeanne & Fagen, 1974; Hunt *et al.*, 1996; Noll *et al.*, 2004; Noll & Wenzel, 2008).

The strong allometric differences detected between castes for nearly all observed traits suggest that caste evolution in *Vespula* wasps may have followed a model proposed by Wheeler (1991). Wheeler suggested that caste evolution begins with casteless individuals that have the same allometric relationships. Morphologically distinct castes begin to form when differently-sized reproductive and subfertile groups evolve. The morphologies of these two groups diverge further because of selection acting upon developmental switches that produce different allometries. Once two discrete size groups are produced, the developmental switches underlying dimorphisms begin to operate earlier in development, allowing the production of castes that differ more in shape (allometry) than in size.

CASTE DIMORPHISM AND INTERCASTE CORRELATIONS

We analysed the association between trait dimorphism and intercaste correlation to detect evidence of past selection on the suite of morphological traits analysed in *V. maculifrons*. We found that caste dimorphism and intercaste correlations were significantly negatively associated (Fig. 3). However, the significance of this correlation was modest ($P = 0.02$). In addition, much of the signal was derived from a single trait: mass. Thus the results from this study must be interpreted with caution.

Regardless, it is noteworthy that a negative correlation between trait dimorphism and intercaste correlation supports the hypothesis that these correlations reflect genetic architectures that constrain the evolution of dimorphism. Moreover, the results suggest

that significant intergroup dimorphism evolves more rapidly if the intergroup correlations for homologous traits are reduced. Our results are consistent with those obtained from analysing traits in sexually, as opposed to caste, dimorphic species (Lande, 1980; Reeve & Fairbairn, 1996, 2001; Ashman, 2003; Bonduriansky & Rowe, 2005; Fairbairn & Roff, 2006; Fedorka, Winterhalter & Mousseau, 2007; Steven, Delph & Brodie, 2007; Bonduriansky & Chenoweth, 2009). However, further analyses performed in other social insects are needed to more fully understand the genetic architecture of caste dimorphism.

CONCLUSIONS

Our findings suggest that caste-specific selection may have acted on several gyne traits in *V. maculifrons*. These results provide an intriguing glimpse into the selective pressures operating on caste morphology in social insects, thereby laying the groundwork for future studies to examine the nature of the distinct selective pressures and functional constraints that have shaped morphological traits in highly caste-dimorphic systems. Additionally, our analyses of dimorphism and intercaste correlations provide a parallel with work performed in sexually dimorphic species, and should help to broaden our understanding of the evolution of phenotypic dimorphisms in different contexts.

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REFERENCES

- Abell AJ, Cole BJ, Reyes R, Wiernasz DC. 1999. Sexual selection on body size and shape in the western harvester ant, *Pogonomyrmex occidentalis cresson*. *Evolution* **53**: 535–545.
- Andersson MB. 1994. *Sexual selection*. Princeton, NJ: Princeton University Press.
- Ashman TL. 2003. Constraints on the evolution of males and sexual dimorphism: field estimates of genetic architecture of reproductive traits in three populations of gynodioecious *Fragaria virginiana*. *Evolution* **57**: 2012–2025.
- Bargum K, Boomsma JJ, Sundström L. 2004. A genetic component to size in queens of the ant, *Formica truncorum*. *Behavioral Ecology and Sociobiology* **57**: 9–16.
- Blackith RE. 1958. An analysis of polymorphism in social wasps. *Insectes Sociaux* **12**: 245–225.
- Bonduriansky R. 2007a. The genetic architecture of sexual dimorphism: the potential roles of genomic imprinting and condition-dependence. In: Fairbairn DJ, Blackethorn WU, Szekely T, eds. *Sex, size, & gender roles: evolutionary studies of sexual size dimorphism*. New York: Oxford University Press, 176–184.
- Bonduriansky R. 2007b. Sexual selection and allometry: a critical reappraisal of the evidence and ideas. *Evolution* **61**: 838–849.
- Bonduriansky R, Chenoweth SF. 2009. Intralocus sexual conflict. *Trends in Ecology and Evolution* **24**: 208–288.
- Bonduriansky R, Rowe L. 2005. Intralocus sexual conflict and the genetic architecture of sexually dimorphic traits in *Prochyliza xanthostoma* (Diptera: Piophilidae). *Evolution* **59**: 1965–1975.
- Cheverud JM. 1984. Quantitative genetics and developmental constraints on evolution by selection. *Journal of Theoretical Biology* **110**: 155–171.
- Cheverud JM. 1988. A comparison of genetic and phenotypic correlations. *Evolution* **42**: 958–968.
- Chippindale AK, Gibson JR, Rice WR. 2001. Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*. *Proceedings of the National Academy of the Sciences USA* **98**: 1671–1675.
- DeHeer CJ. 2002. A comparison of the colony-founding potential of queens from single- and multiple-queen colonies of the fire ant *Solenopsis invicta*. *Animal Behaviour* **64**: 655–661.
- Diniz JAF, Vonzuben CJ, Fowler HG, Schlindwein MN, Bueno OC. 1994. Multivariate morphometrics and allometry in a polymorphic ant. *Insectes Sociaux* **41**: 153–163.
- Elmes GW. 1991. Mating strategy and isolation between 2 forms, macrogyna and microgyna, of *Myrmica ruginodis* (Hymenoptera: Formicidae). *Ecological Entomology* **16**: 411–423.
- Fairbairn DJ. 1997. Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. *Annual Review of Ecology and Systematics* **28**: 659–687.
- Fairbairn DJ, Roff DA. 2006. The quantitative genetics of sexual dimorphism: assessing the importance of sex-linkage. *Heredity* **97**: 319–328.
- Falconer DS, Mackay TFC. 1996. *Introduction to quantitative genetics*. Essex, UK: Longman Group Limited.
- Fedorka KM, Winterhalter WE, Mousseau TA. 2007. The evolutionary genetic of sexual size dimorphism in the cricket *Allonemobius socius*. *Heredity* **99**: 218–223.
- Fjerdingstad EJ. 2005. Control of body size of *Lasius niger* ant sexuals – worker interests, genes and environment. *Molecular Ecology* **14**: 3123–3132.
- Fjerdingstad EJ, Crozier RH. 2006. The evolution of worker caste diversity in social insects. *American Naturalist* **167**: 390–400.
- Fraser VS, Kaufmann B, Oldroyd BP, Crozier RH. 2000. Genetic influence on caste in the ant *Camponotus consobrinus*. *Behavioral Ecology and Sociobiology* **47**: 188–194.

- Goodisman MAD, Kovacs JL, Hoffman EA. 2007a.** The significance of multiple mating in the social wasp *Vespula maculifrons*. *Evolution* **61**: 2260–2267.
- Goodisman MAD, Kovacs JL, Hoffman EA. 2007b.** Lack of conflict during queen production in the social wasp *Vespula maculifrons*. *Molecular Ecology* **16**: 3063–3065.
- Goodisman MAD, Kovacs JL, Hunt BG. 2008.** Functional genetics and genomics in ants (Hymenoptera: Formicidae): the interplay of genes and social life. *Myrmecological News* **11**: 107–117.
- Greene A. 1991.** *Dolichovespula* and *Vespula*. In: Ross KG, Matthews RW, eds. *The social biology of wasps*. Ithaca, NY: Cornell University Press, 263–305.
- Heinze J. 1989.** Alternative dispersal strategies in a North American ant. *Naturwissenschaften* **76**: 477–478.
- Hoffman EA, Goodisman MAD. 2007.** Gene expression and the evolution of phenotypic diversity in social wasps. *BMC Biology* **5**: Art. 23.
- Hoffman EA, Kovacs JL, Goodisman MAD. 2008.** Genetic structure and breeding system in a social wasp and its social parasite. *BMC Evolutionary Biology* **8**: 239.
- Humphries JM, Bookstein FL, Chernoff B, Smith GR, Elder RL, Poss SG. 1981.** Multivariate discrimination by shape in relation to size. *Systematic Zoology* **30**: 291–308.
- Hunt JH, Schmidt DK, Mulkey SS, Williams MA. 1996.** Caste dimorphism in the wasp *Epipona guerini* (Hymenoptera: Vespidae; Polistinae, Epinonini): further evidence for larval determination. *Journal of the Kansas Entomology Society* **69**: 362–369.
- Jeanne RL, Fagen R. 1974.** Polymorphism in *Stelopolybia areata* Hymenoptera Vespidae. *Psyche* **81**: 155–166.
- Johnson EL, Cunningham TW, Marriner SM, Kovacs JL, Hunt BG, Bhakta DB, Goodisman MAD. 2009.** Resource allocation in a social wasp: effects of breeding system and life cycle on reproductive decisions. *Molecular Ecology* **16**: 2589–2595.
- Jolicoeur P. 1963.** The multivariate generalization of the allometry equation. *Biometrics* **19**: 497–499.
- Kovacs JL, Hoffman EA, Goodisman MAD. 2008.** Mating success in the polyandrous social wasp *Vespula maculifrons*. *Ethology* **114**: 340–350.
- Kovacs JL, Hoffman EA, Marriner SM, Rekau JA, Goodisman MAD. 2010.** Environmental and genetic influences on queen and worker body size in the social wasp *Vespula maculifrons*. *Insectes Sociaux* **57**: 53–65.
- Lande R. 1980.** Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Proceedings of the National Academy of the Sciences USA* **78**: 3721–3725.
- Linksvayer TA, Wade MJ. 2009.** Genes with social effects are expected to harbor more sequence variation within and between species. *Evolution* **63**: 1685–1696.
- Macdonald JF, Matthews RW. 1981.** Nesting biology of the eastern yellowjacket, *Vespula maculifrons* (Hymenoptera, Vespidae). *Journal of the Kansas Entomological Society* **54**: 433–457.
- Mirth CK, Riddiford LM. 2007.** Size assessment and growth control: how adult size is determined in insects. *Bioessays* **29**: 344–355.
- Molina Y, Harris RM, O'Donnell S. 2009.** Brain organization mirrors caste differences, colony founding and nest architecture in paper wasps (Hymenoptera: Vespidae). *Proceedings of the Royal Society of London B* **276**: 3345–3351.
- Nijhout HF, Wheeler DE. 1996.** Growth models of complex allometries in holometabolous insects. *American Naturalist* **48**: 40–56.
- Noll FB, Wenzel JW. 2008.** Caste in the swarming wasps: 'queenless' societies in highly social insects. *Biological Journal of the Linnean Society* **93**: 509–522.
- Noll FB, Wenzel JW, Zucchi R. 2004.** Evolution of caste in neotropical swarm-founding wasps (Hymenoptera: Vespidae; Epiponini). *American Museum Novitates* **3467**: 1–24.
- O'Donnell S. 1998.** Reproductive caste determination in eusocial wasps (Hymenoptera: Vespidae). *Annual review of entomology* **43**: 323–346.
- Oster GF, Wilson EO. 1978.** *Caste and ecology in the social insects*. Princeton, NJ: Princeton University Press.
- Price T, Langen T. 1992.** Evolution of correlated characters. *Trends in Ecology and Evolution* **7**: 307–310.
- Reeve JP, Fairbairn DJ. 1996.** Sexual size dimorphism as a correlated response to selection on body size: an empirical test of the quantitative genetic model. *Evolution* **50**: 1927–1938.
- Reeve JP, Fairbairn DJ. 2001.** Predicting the evolution of sexual size dimorphism. *Journal of Evolutionary Biology* **14**: 244–254.
- Ross KG. 1985.** Aspects of worker reproduction in 4 social wasp species (Insecta, Hymenoptera, Vespidae). *Journal of Zoology* **205**: 411–424.
- Roux EA, Roux M, Korb J. 2009.** Selection on defensive traits in a sterile caste – caste evolution: a mechanism to overcome life-history trade-offs? *Evolution and Development* **11**: 80–87.
- Rüppell O, Heinze J, Hölldobler B. 1998.** Size-dimorphism in the queens of the North American ant *Leptothorax rugatulus* (Emery). *Insectes Sociaux* **45**: 67–77.
- Schoning C, Kinuthia W, Franks NR. 2005.** Evolution of allometries in the worker caste of *Dorylus* army ants. *Oikos* **110**: 231–240.
- Spradbery JP. 1973.** *Wasps: an account of the biology and natural history of solitary and social wasps*. Seattle, WA: University of Washington Press.
- Steven JC, Delph LF, Brodie ED. 2007.** Sexual dimorphism in the quantitative-genetic architecture of floral, leaf, and allocation traits in *Silene latifolia*. *Evolution* **61**: 42–57.
- Strauss RE. 1987.** On allometry and relative growth in evolutionary studies. *Systematic Zoology* **36**: 72–75.
- Tschinkel WR, Mikheyev AS, Storz SR. 2003.** Allometry of workers of the fire ant, *Solenopsis invicta*. *Journal of Insect Science* **3**: 2.
- Turillazzi S, Francescato E, Baldini Tosi A, Carpenter JM. 1994.** A distinct caste difference in *Polybioides tabidus* (Fabricius) (Hymenoptera: Vespidae). *Insectes Sociaux* **41**: 327–330.
- West GB, Brown JH, Enquist BJ. 1997.** A general model for the origin of allometric scaling laws in biology. *Science* **276**: 122–126.

- Wheeler DE. 1986.** Developmental and physiological determinants of caste in social Hymenoptera: evolutionary implications. *American Naturalist* **128**: 13–34.
- Wheeler DE. 1991.** The developmental basis of worker caste polymorphism in ants. *American Naturalist* **138**: 1218–1238.
- Whitlock MC. 2005.** Combining probability from independent tests: the weighted Z-method is superior to Fisher's approach. *Journal of Evolutionary Biology* **18**: 1368–1373.
- Wiernasz DC, Cole BJ. 2003.** Queen size mediates queen survival and colony fitness in harvester ants. *Evolution* **57**: 2179–2183.
- Wilson EO. 1953.** The origin and evolution of polymorphism in ants. *Quarterly Review of Biology* **28**: 136–156.
- Wilson EO. 1971.** *The insect societies*. Cambridge, MA: Harvard University Press.