QUEEN-SPECIFIC SELECTIVE PRESSURES AND CASTE DIMORPHISM IN THE

SOCIAL WASP *V. MACULIFRONS*

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QUEEN-SPECIFIC SELECTIVE PRESSURES AND CASTE DIMORPHISM IN THE

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To Mama…

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LIST OF ABBREVIATIONS

3TL and T3L	Third Tergum Length, a prominent abdominal segment
AL	Mean Antennal Length
ABI	Applied Biosystems Incorporated
ANOVA	Analysis of Variance
ANCOVA	Analysis of Covariance
BL	Total Body Length
CD	Caste Dimorphism
CL	Length of the distal Cell of the right wing
CV	Coefficient of Variation
df	Degrees of Freedom
G	Gyne, a pre-reproductive female
GL	Gaster Length, the measure of the abdomen without the first tegum
HTL	Head to Thorax Length
H^2	Broad sense heritability, extent to which individuals' phenotypes are determined by genotypes, V_G/V_P
h^2	Narrow sense heritability, extent to which phenotypes are determined by the genes transmitted from the parent, V_A/V_P
k_{e3}	Effective paternity
k	Multivariate allometric coefficient
M	Male
M	Mated
PC	Principal Component
PC1	Principal Component 1
PC2	Principal Component 2
PCR	Polymerase Chain Reaction

r^2	Pearson's correlation coefficient
R_s and r_s	Spearman's rank correlation coefficient
SD	Standard Deviation
SE	Standard Error
SMA	Standardized Major Axis
TL	Thorax Length
TW	Thorax Width, measure from the left to the right tegula
U	Unmated
V_G	Genetic Variance
V_P	Total Phenotypic Variance
V_D	Dominance genetic Variance
V_{EC}	Common Environmental Variance
V_{EW}	Environmental Variance shared by offspring Within patrilines

SUMMARY

The reproductive division of labor between workers and queens is one of the hallmarks of highly social insects (ants, termites, some bees, and some wasps). Within social insect colonies, sterile workers are responsible for foraging, nest building, and brood care, while queens act as the primary egg-layers. These functional and reproductive caste differences are often accompanied by pronounced physiological and morphological distinctions. The phenotypic differences displayed by queen and worker castes are particularly remarkable considering that caste is environmentally, rather than genetically, determined. Because both queen and worker phenotypes can result from the same genotype, there exists the potential for intralocus genetic conflict between castes, particularly when homologous traits (i.e. traits expressed in both castes) are highly dimorphic. Therefore, when studying the evolution of social insect caste dimorphism, it is important to take into consideration the genetic architecture underlying phenotypic expression as well as the selective pressures that have shaped caste morphology.

This dissertation presents the results of four studies that investigated factors affecting caste morphology in the social wasp *Vespula maculifrons*. The first two studies (chapters 2 and 3) focused on identifying queen morphological traits that were positively associated with queen fitness (mating success and overwintering survival), and would therefore be subject to selection. Queen length, specifically gaster length (the measure of the abdomen not including the first abdominal segment), was positively associated with overwintering survival and was consistently associated with mating success. Therefore, queens with longer gasters survive overwintering longer and generally mate at higher frequencies than queens with shorter gasters. Both of these findings suggest that queen gaster length is under selection during two life-history events, mating and

overwintering, in which workers do not participate. These findings provide empirical support for the adaptive evolution of a caste dimorphic trait.

The third and fourth studies, presented in chapters 4 and 5, used classical quantitative genetic and morphological analyses to examine the genetic architecture underlying caste dimorphism in *V. maculifrons*. I determined which traits were under caste-specific selection by analyzing trait allometries and the levels of genetic control, variation, and dimorphism of traits between castes. Little genetic variation for morphological trait size was detected for most worker and queen traits, suggesting a strong influence of environment on phenotypic variation. However, several queen traits, including gaster length, possessed significant genetic variability (i.e. heritabilities). These results suggest that traits possessing significant heritabilities have not experienced strong directional selection. However, other components of variation, including epistatic and indirect genetic effects, can generate significant heritabilities. These effects are often associated with traits that have undergone directional selection, and therefore may have influenced the significant queen heritabilities detected in our study. Additionally, analyses of trait allometries indicated that several queen traits (mass, thorax width and length) were under queen-specific selection. The relationship between thorax length, gaster length, and overall body size is further evidence of selection on length in queens.

Overall, these studies provide evidence for the importance of queen-specific selection in the evolution of caste dimorphism. When placed in the broader context of caste evolution, they point to the importance of life-history in shaping the genetic architecture underlying caste dimorphism. Therefore, this work further elucidates how natural selection operates in highly social systems.

CHAPTER ONE

THE EVOLUTION OF CASTE AND CASTE DIMORPHISM IN SOCIAL INSECTS

Social insects represent a highly diverse and ecologically successful animal group. Examples of their ecological dominance include the estimated 90% of all dead insects that ants are estimated to scavenge (O'TOOLE 1993) and the 97% of crops pollinated by honey bees and bumble bees in the U.S. (BORROR *et al.* 1989). Their ecological success is no doubt linked to their astounding abundance. In tropical rain forests, social insects compose an estimated 80% of total insect biomass (FITTKAU & KLINGE, 1973), and in rain forests and savannahs, the biomass of ants and termites outweighs that of all mammalian fauna (OTTO 2008). Despite the tremendous ecological and numerical success of social insects, sociality has evolved in only two insect orders: the Isoptera (termites) and the Hymenoptera (ants, bees, and wasps; but see COSTA 2006), and social insects comprise only ~2% of described insect species (ARNETT 1985). One of the key distinguishing characteristics of social insects that has played a large role in their ecological dominance, is a reproductive division of labor between sterile workers and fertile reproductives. The question of how an entire group of sterile, non-reproductive individuals (i.e. workers) could evolve and then be maintained within a species has long been central to social insect research.

Kin Selection & the Problem of Altruism

In 1859 Charles Darwin in *On the Origin of Species* was puzzled by the presence of the sterile, morphologically distinct workers found in honeybee colonies. Darwin noted that this "one special difficulty" could have been "fatal" to his theory of evolution by natural selection. However, he realized that these sterile individuals were found in social

groups and therefore would be "profitable to the community", which would aid in the maintenance of a sterile class within the colony (DARWIN 1859). Of course, this observation is too simplistic to fully explain the evolution and persistence of a sterile worker caste. Though, as Darwin originally noted, the social and communal nature of most altruistic behaviors has been a key attribute of the evolutionary theories that followed.

It is important to first specifically define the dilemma posed by social insect castes. Under the theory of natural selection, an individual's fitness is dependent upon its reproductive success. Within most hymenopteran social insect colonies, queens are the only individuals that reproduce, while workers are sterile and aid in the rearing of the queen's brood (WILSON 1971). Therefore social insect workers exhibit altruism, i.e. one actor (workers) help another actor (queens) to increase their reproductive fitness at a seeming cost to their own fitness (WEST 2007). Due to the reproductive fitness loss associated with altruistic behaviors, the question of how sterile workers attained fitness benefits presented a potential challenge to evolutionary biologists.

In 1964, a theoretical framework was formed to explain the seemingly paradoxical evolution of altruism; these theories became known as Hamilton's rule or kin selection theory (HAMILTON 1964a; 1964b; MAYNARD SMITH 1964). Under kin selection theory, an individual's overall fitness, or inclusive fitness, is composed of both direct and indirect fitness effects (HAMILTON 1964a; 1964b; GRAFEN 1984). Direct fitness is gained by producing offspring (WEST 2007). While indirect fitness is gained by directing helping behaviors towards others that share the same "helping" behavior allele (HAMILTON 1964a; 1964b). Under the narrow definition of kin selection, when alleles are identical by descent, an individual is still passing on its alleles, including the "helping" behavior allele, to the next generation by helping a close relative reproduce, albeit indirectly (WEST

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2007). In this way, if indirect fitness is high enough, sterile workers can attain inclusive fitness, and the "helping" allele is passed on to the next generation. Therefore, kin selection theory provides a mechanism by which costly "helping" behaviors, such as altruism, and therefore sterile worker castes, can evolve.

Evolution of Group-Living and Sociality

Though kin selection theory can explain how sterile worker castes and their altruistic brood care behaviors evolved, other theories are needed to explain how the wide range of complex social behaviors exhibited by modern-day social insects arose. Two different, and not mutually exclusive, pathways have been proposed for the evolution of sociality and group-living in social insects. The *parasocial* route proposed by LIN & MICHENER (1972) begins with groups of unrelated females of the same generation sharing nests due to the mutualistic advantages of cooperative nest guarding and building. Group-living then proceeds through a semisocial organizational stage in which individuals cooperatively rear offspring and some females begin to exhibit reproductive dominance over others. Finally, a highly social organization can form, in which there is strong reproductive dominance among female group members, and ultimately female caste dimorphism between reproductives and helpers can evolve. This mutualistic model of social evolution faced several theoretical hurdles, particularly due to the lack of relatedness among the founding females which precludes the action of kin selection and therefore the evolution of altruistic behaviors (ALEXANDER 1974; CROZIER 1979). Due to the theoretical problems with Michener's original theory, WEST-EBERHARD (1975) proposed a variation of the parasocial route, known as the *polygynous family hypothesis*, in which several related females form polygynous (multiple queen) associations that are maintained through several generations, eventually resulting in mother-offspring

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associations. From these small polygynous associations, highly social groups exhibiting reproductive dominance and even dimorphism can evolve.

Alternatively, the *subsocial* route to sociality, originally proposed by WHEELER (1922), begins with nest establishment by a single queen (or monogyny), and then progresses to small mother-offspring associations in which offspring aid in the rearing of their siblings over multiple generations. As in the parasocial model, highly social colonies with strong reproductive dominance by the mother and even pronounced worker and queen dimorphism can arise from these simple mother-offspring associations. Both the parasocial and subsocial theories provide a framework in which to test hypotheses concerning the evolution of the wide range of social behaviors and syndromes exhibited by modern social insects.

Understanding the evolutionary origins of group-living and sociality has been the focus of many evolutionary and behavioral studies for the last several decades (e.g. WEST-EBERHARD 1975; LIN & MICHENER 1973; WHEELER 1922; CARPENTER 1991; DANFORTH 2002). Unfortunately, the model social insects; honey bees, ants and termites, can provide limited insights into the evolutionary origins of sociality due to the limited range of social behaviors found with these groups (HÖLLDOBLER & WILSON 1990; DANFORTH 2002). However, two other groups of hymenoptera, the bee family Halictidae (WILSON 1971) and the wasp family Vespidae (GREENE 1991), do exhibit the variable levels of sociality, including solitary living species, necessary to analyze the origins of sociality. Two studies, one performed in Halictidae bees (DANFORTH 2002) and the other in Vespidae wasps (CARPENTER 1991), tested the parasocial and subsocial models by mapping social behaviors onto phylogenetic trees of the two families. Interestingly, these studies provided evidence for both models. DANFORTH (2002) found no relationship between communal and semisocial nesting and the evolutionary origins of highly social

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behavior in Halictidae bees, providing evidence for the subsocial model in this family. While, CARPENTER (1991) found that the most basal vespid wasp subfamilies exhibit only parasocial associations of sisters, thereby providing support for the parasocial model of social evolution in wasps. Of course, these findings do not negate each other, but rather highlight the importance of the different taxonomic and ecological circumstances under which group-living, sociality, and caste have evolved within the hymenoptera.

Evolution of Caste Dimorphism

As described above, the presence of worker and queen castes is a key characteristic shared by all social insects. Castes are primarily defined by their reproductive roles within colonies, with queens acting as the primary reproductives and workers producing few, if any, offspring (WILSON 1971). Often this reproductive division is accompanied by other behavioral, physiological and morphological distinctions. For instance, workers are more likely to perform "risky" tasks such as colony defense and foraging (GRECHKA & KIPYATKOV 1983; YANEGA 1992; O'DONNELL 1998), whereas queens are often responsible for colony founding (STRAMBI 1985). Additionally, there are often differences in the reproductive physiology of workers and queens. These differences include insemination status, ovary development, and the amount of fat stored; the presence of all these traits are indicative of reproductive or queen status within most social insects (O'DONNELL 1998). Finally, workers and queens in many species exhibit caste-specific morphologies which can often take the form of pronounced caste dimorphism.

As mentioned earlier, Darwin was puzzled by his observations of social insect sterile worker castes. Not only did their seemingly selfless behavior present problems for the theory of natural selection, but their caste-specific morphologies, especially the

transmission and evolution of worker morphologies, provided yet another challenge to his theories of evolution (DARWIN 1859). Indeed there are still many unanswered questions surrounding the evolution of caste dimorphism, ranging from those focused on differential gene expression between the castes (reviewed by ROBINSON *et al.* 2008) to those trying to understand the ecological conditions under which caste dimorphism evolved (reviewed by O'DONNELL 1998; BOURKE 1999).

In an effort to explain the varying levels of sociality exhibited throughout the social hymenoptera, BOURKE (1999) analyzed the positive association between colony size and social complexity, and also provided a framework in which to examine the evolution of caste dimorphism. The positive correlation between colony size and caste dimorphism is thought to be due to the inverse relationship between colony size and worker reproductive potential (BOURKE 1999). As colony size increases, competition for direct reproductive fitness also increases among potential reproductives (i.e. non-primary reproductives) and therefore the chances of becoming the primary reproductive within the colony decreases. Conversely among smaller colonies, non-reproductive individuals may still be able to attain direct reproductive fitness either through a secondary reproductive status or by replacing the reproductive queen by active queen usurption or upon the death of the queen. Therefore, in smaller colonies, workers would be expected to keep their reproductive options open for as long as possible in case the chance to become the primary reproductive does arise. The continued possibility of direct reproduction would result in caste determination occurring during adulthood (post-imaginal caste determination). Due to the growth patterns of hymenopteran insects, this delay in caste determination would preclude the evolution pronounced dimorphisms between workers and queens (WHEELER 1986a). Hence, pre-imaginal caste determination, and therefore well-defined caste-specific morphologies, are expected to

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evolve only in instances where the attainment of direct reproductive fitness is extremely rare for non-reproductives, (i.e. in large, complex colonies; BOURKe 1999).

Pre-imaginal caste determination is essential to the production of highly dimorphic queen and worker castes (WHEELER 1986a; O'DONNELL 1998). Hymenopteran insects are holometabolous and therefore do not molt during their adulthood. Once an individual reaches its adult stage, its hardened (sclerotized) body parts do not change size. Therefore, any differences between adults in the sizes of hard body parts is due to developmental differences that occurred during immature life stages (WHEELER 1986a). This means that early caste determination, which is more likely to occur in complex large colony size species, is the only route by which pronounced caste dimorphism can evolve (BOURKE 1999).

Indeed the extreme caste dimorphisms displayed by some social insect species is truly astounding, especially when we consider that caste is environmentally rather than genetically determined in most social insects. How these caste-specific morphologies can evolve without genetic divergence (i.e. with shared worker and queen genotypes) has become the focus of many recent molecular studies, and there is strong evidence for the action of caste-specific gene expression in shaping the morphological, physiological, and behavioral distinctions between workers and queens (EVANS & WHEELER 1999; 2001; SCHARF *et al.* 2003; GROZINGER *et al.* 2007; SMITH *et al.* 2008; ELANGO *et al.* 2009). Though at the heart of all these investigations, are the selective pressures that have operated on worker and queen morphologies.

Caste Dimorphism & Queen-Specific Selective Pressures

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Much of the previous work examining caste dimorphism in social insects has sought to explain the evolution of worker adaptations and morphology (OSTER & WILSON 1978; WHEELER 1991; FJERDINGSTAD & CROZIER 2006). For example, much emphasis has been placed on the decrease in overall worker body size in comparison to queen body size (JEANNE 1980; O'DONNELL 1998). This focus on worker morphology is largely due to the derived life-history of sub-fertile workers (WEST-EBERHARD 1975). However, in social wasps, several queen morphological traits have been found to be disproportionately large or small relative to overall body size, while worker morphological traits, as well as queen morphological traits in non-dimorphic species, tend to be proportional to overall body size (BLACKITH 1958; EICKWORT 1969; JEANNE & FAGEN 1974; LITTE 1977; TURILLAZZI & PARDI 1977; CHANDRASHEKARA & GADAGKAR 1991; TURILLAZZI *et al.*1994). The disproportional trait sizes exhibited by queens in dimorphic species suggest that selective pressures altered the growth rates of these morphological traits during development, and that therefore they are under caste-specific selection during pre-imaginal caste determination.

I propose that though worker-specific selective pressures have played a role in shaping the pronounced caste dimorphisms found in many social insects, these selective pressures are largely diminished once "fixed", obligatory worker sterility is attained. Worker-specific selective pressures may also be buffered by the indirect method through which workers attain fitness (i.e. inclusive fitness; GADAGKAR 1997). Additionally, I suggest that further caste dimorphism, once obligate worker sterility evolves, is largely driven by queen-specific selection due to selection events such as mating, overwintering, colony founding, and reproduction which are undergone solely by members of the queen caste.

This dissertation seeks to identify the selective pressures that have influenced the evolution of caste dimorphism, particularly queen morphology, in the social wasp *Vespula maculifrons*, a species that among social wasps is highly caste dimorphic. *Vespula maculifrons* exhibits clear sexual and caste dimorphisms, with queens being much larger than both males and workers. *Vespula maculifrons* colonies are annual and established by a single queen, which emerges from overwintering in the spring, like those of most other social wasps in the sub-family *Vespinae*. *Vespula maculifrons* queens mate with multiple males (range in queen mate number of 3-9; ROSS 1985; GOODISMAN *et al.* 2007b), though queen mate number is variable throughout the *Vespidae*, with most species effectively mating with only one male (GREENE 1991). For the first several months after establishment, the colony produces only workers, which are reared in worker comb. Gynes (pre-reproductive queens) are produced toward the end of the colony cycle in specialized queen comb. Additionally, *V. maculifrons* colonies produce abundant workers and new queens each year, thus providing the material necessary for extensive morphological and genetic studies.

This dissertation is composed of four experimental studies exploring the evolution of caste dimorphism, presented in chapter 2 through 5. In chapters 2 and 3, I focus on identifying queen morphological traits associated with fitness events specific to queen life-histories, namely mating success and overwintering survival. By identifying these traits, we are better able to identify the functional causes underlying caste-specific selection on queen morphology. In chapters 4 and 5, I present work that through analyses of worker and queen morphologies, identifies which worker and queen morphological traits have experienced caste-specific selection. In the final chapter (chapter 6), I synthesize the results of these four studies in order to provide a general overview of the effect of queen-specific selection on caste dimorphism in *V. maculifrons*.

Overall, my dissertation stresses the importance of queen-specific selection events and the selective pressures they produce on the evolution of caste dimorphic traits.

CHAPTER 2[1]

MATING SUCCESS IN THE POLYANDROUS SOCIAL WASP *VESPULA MACULIFRONS*

Abstract

The mating decisions made by social insect males and females profoundly affect the structure of colonies and populations. However, few studies have used experimental approaches to understand mating behavior and mate choice in social insect taxa. This study investigated mating success in the polyandrous social wasp *Vespula maculifrons*. Mating trials were designed to test predictions that characteristics of body size and colony-of-origin would affect mating success. We first investigated if size differences existed among individuals and found that males from different colonies differed significantly in the size of nine morphological traits. However, male trait size was not significantly associated with male mating success. In contrast, females from different colonies differed significantly in only six of the nine measured traits, and four of these traits were associated with successful mating behaviors. Specifically, the correlated traits of gaster length, third tergum length, antennal length, and total length were positively associated with female mating success. Thus long females experience mating advantages over females that are short. We also found that males and females from one particular colony displayed significantly greater mating activity than individuals from other colonies. Thus the colony from which individuals originate plays an important role in determining mating success. Finally, our experiments failed to detect any evidence of

nestmate avoidance during the mating trials. Overall, our data suggest that social insect reproductives may experience differential mating success based on their phenotype or developmental environment.

Introduction

The females of many species mate with multiple males (polyandry; BIRKHEAD & MØLLER 1998; ARNQVIST & NILSSON 2000; EBERHARD 2000; JENNIONS & PETRIE 2000). However, polyandry is relatively rare among social hymenopteran insects (ants, some bees and wasps; STRASSMANN 2001; BROWN & SCHMID-HEMPEL 2003; KRONAUER *et al*. 2004). Nevertheless, polyandry has profound and far-reaching consequences on social insect ecology and evolution because of the effects that multiple mating ultimately have on colony structure and diversity (CROZIER & FJERDINGSTAD 2001; STRASSMANN 2001). Both polyandrous and monandrous females may choose particular males as mates based on the direct or indirect benefits that the males provide. For example, females may mate with males providing substantial direct benefits, such as large nuptial gifts. However, social insect males apparently do not provide these types of direct benefits (FJERDINGSTAD & KELLER 2004). Thus, it is posited that females in polyandrous social insect taxa mate multiply to obtain indirect benefits such as greater genetic variability among offspring, which may be associated with increased colony fitness (PALMER & OLDROYD 2000; BROWN & SCHMID-HEMPEL 2003). If females mate multiply to obtain indirect genetic benefits, female mate choice may be based on male traits that reflect some aspect of high fitness. These male fitness characteristics may be male specific ornaments or large size, which demonstrate a male's genetic quality or health status (ANDERSSON 1994; ANDERSSON & SIMMONS 2006).

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Males may also practice mate choice (BOOMSMA & RATNIEKS 1996; BAER 2003; BOOMSMA *et al.* 2005). In general, male choosiness is likely to evolve in systems in which (1) males invest valuable or limited resources during mating, (2) there is a high variation in female quality, or (3) the costs of mate search and assessment are low (BONDURIANSKY 2001). For many species of social insects these conditions may hold true. Males of most hymenopteran social insect species undergo spermatogenesis only during development and therefore have a limited number of sperm to invest in their lifetime (ANDERSON *et al*. 2003; BAER 2003, BOOMSMA *et al*. 2005; but see HEINZE & HÖLLDOBLER 1993). Additionally, females vary significantly in their ability to overwinter and successfully establish colonies (GERLOFF & SCHMID-HEMPEL 2005). Moreover, for lekking species, the costs of mate location may be low. Thus, social insect males may select females that display characteristics associated with fecundity, such as large body size (BONDURIANSKY 2001).

Despite the unusual and important implications of mate choice to social systems, little is known about mating decisions in polyandrous social insect taxa. Studies are limited due to the difficulty of observing mating activity under natural conditions. Furthermore, many social insects do not readily mate under laboratory conditions (CROZIER & FJERDINGSTAD 2001). The wasp *Vespula maculifrons* is one of the few polyandrous social insect taxa that will mate readily and repeatedly under laboratory conditions. *V. maculifrons* is found in the eastern United States and builds large, annual, subterranean nests (MACDONALD & MATTHEWS 1981; GREENE 1991). *V. maculifrons* exhibits clear sexual and caste dimorphisms, with queens being much larger than both males and workers. A new *V. maculifrons* colony is established by a single, multiply-mated queen, which emerges from overwintering in the spring. For the first several months after establishment, the colony produces only workers. Gynes (pre-reproductive

queens) are produced toward the end of the colony cycle in specialized queen comb. Males are also produced during this time, though they are generally found in the nest before the emergence of gynes (GREENE 1991). Nevertheless, both sexually mature males and females can be found in the nest simultaneously.

Upon reaching sexual maturity, both males and gynes leave the nest to partake in mating flights. Based on limited data, it is believed that *V. maculifrons* males lek near prominent vegetation with few competitive interactions occurring between males (SPRADBERY 1973; ROSS & CARPENTER 1991). Gynes typically mate with multiple males (GOODISMAN *et al*. 2007a; 2007b; KOVACS & GOODISMAN 2007). Males are also capable of mating with multiple females under laboratory conditions (ROSS 1983). Mating behavior has been described as occurring in a four part sequence; males mount, insert, assume a free-hanging position, and then form an S-position with the gyne during a successful mating attempt (ROSS 1983).

In this study, we designed sets of controlled mating experiments to test the effects of size and colony-of-origin on male mating success. Specifically, we wanted to know (1) if large size was associated with male mating success, as is the case in other animal taxa (BLANCKENHORN 2005), (2) if individuals avoided mating with their nestmates, which is expected if consanguineous matings result in low fitness (ZEH & ZEH 2003), and (3) if individuals from particular colonies were more successful in obtaining matings than those from other colonies (i.e., an influence of colony-of-origin on mating success). We conclude by addressing how our results further our understanding of the consequences of mating success in social insects.

Methods

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Collection and maintenance of samples

Six underground *V. maculifrons* colonies were collected from locations around

Atlanta, Georgia U.S.A. between September and October of 2004. Males and gynes

were collected from four and five colonies, respectively. To assure that gynes were

virgins at the time of the mating trials, queen comb containing pupal gynes was

separated from the rest of the colony, and gynes were allowed to eclose from this comb

in containers separate from males. All gynes used in experiments were capable of flight

indicating that they were sexually mature (ROSS 1983). Males typically did not eclose

from worker comb under laboratory conditions. Therefore males that were present in the

colony at the time of collection were used for mating trials.

Mating experiments

Ninety mating trials designed to test factors influencing male mating success

were conducted. In each forty-five minute trial, one female was presented with two

males belonging to different size classes (large versus small). Additionally, mating trials

were set up such that the males could (1) both be from the same colony as the gyne, (2)

both be from different colonies than the gyne, or (3) one male be from the same colony

as the gyne and the other male be from a different colony than the gyne. Males within

trials were not size matched so in all cases one male was larger than the other (Fig. 2.1).

The two males were marked with different colored dots of paint on their thoraces. Our

pretrial experimentation indicated that these small paint dots, although clearly visible to

the observer, did not alter the behavior of the wasps. In addition, marking colors were

chosen in advance of the trials, so the observer did not know the colony-of-origin of the

marked males.

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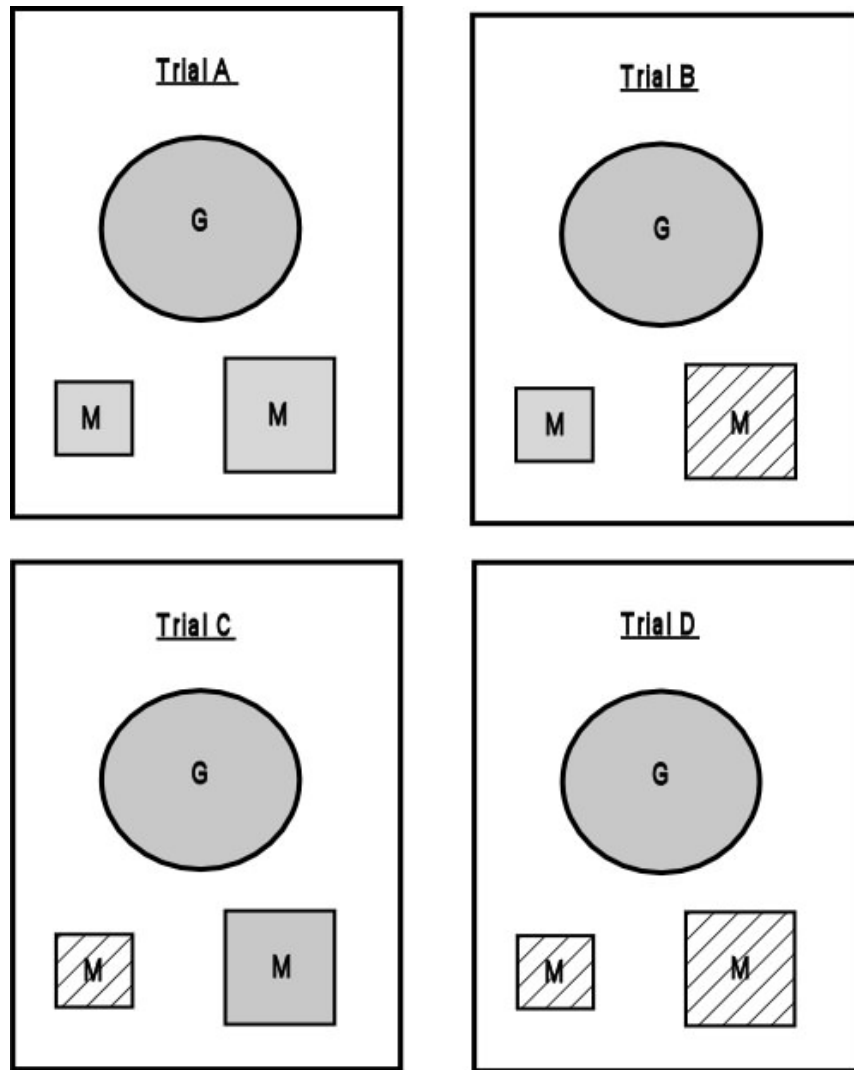
Figure 2.1: Examples of the four different types of experimental mating trials used to test the importance of size, inbreeding avoidance, and colony-of-origin in the polyandrous social wasp *V. maculifrons*. Sizes of squares denote relative sizes of males used in trials (either large or small). Trial A - both males are from the same colony as the gyne. Trial B - the small male is from the same colony as the gyne and a large male is from a different colony. Trial C - the small male is from a different colony than the gyne and the large male is from the same colony as the gyne. Trial D - both males are from a different colony as the gyne. G = Gyne. M = Male.

We observed and recorded the number of times a male mounted a female and the length of each of those mountings. Additionally, we scored putatively successful matings (insertions) based upon the characteristic S-shape that the male and gyne form after insertion (ROSS 1983). Sperm transfer was confirmed through dissections of several gynes.

After trials were completed, all gynes and males were preserved in 95% ethanol. Subsequently, gynes and males were dried overnight at 60° C, and the dry mass of each individual was obtained. Digital photographs of all individuals were taken in two positions: a dorsally facing position and a position with the right wing extended. A Zeiss© millimeter micrometer was included in each photo to allow for size calibration. For all individuals, we measured the length from the top of the head to the end of the thorax (HTL), thorax width (TW; from the inside of the left wing tegula to the inside of the right wing tegula), thorax length (TL), gaster length (GL), total body length (BL), mean antennal length (AL), length of the third tergum (T3L; tergum 3 is a large prominent tergum that is found close to the petiole), and length of the distal cell on the right wing (CL, the distal cell has been used as a general measure of size in *Vespula*; SPRADBERY 1973; ROSS 1983) using tools within the Canvas 9.0.4 computer package.

Morphometrics

We tested for the presence of significant differences in size measurements and dry mass for males and gynes sampled from different colonies using one-way ANOVA. We determined if morphological variables were significantly correlated within sexes using Spearman's rank correlation coefficient (r_s). Principal component analysis of correlations was performed with all nine measured traits for both males and gynes. All statistical analyses were conducted using JMP 3.2.6.

Mating success and size

We determined if male trait size was associated with mating success. Specifically, the trait value for the male that inserted less was subtracted from the trait value for the male that inserted more. A negative value indicated that the male that inserted less had a larger trait; a positive value indicated that the male that inserted more had the larger trait. The numbers of negative and positive results were then analyzed for significance using a two-tailed sign test.

In addition to analyzing data from all trials, we evaluated the importance of size to male mating success in several data subsets. The first subset included trials in which at least one male displayed activity. We included this subset because inactivity may have been due to time of day, physical well being of the males or gynes, or receptiveness of the gyne used for the trial. The second data subset included cases in which only one male exhibited all mating behaviors. In these cases, we were interested in determining if there were greater differences in male trait size in trials in which all mating activities were performed by one male than in cases where both males showed mating activity.

We next turned our attention to investigating if gyne trait size was associated with gyne mating success. We predicted that large gynes would be particularly likely to be mated. To test our prediction, we used t-tests to determine if the sizes of mated and unmated gynes differed significantly.

Inbreeding avoidance

To test for inbreeding avoidance, we counted the number of times males from the same colony as the gyne mounted or inserted during trials, and compared this to the

number of times males from a different colony as the gyne mounted or inserted using a sign test. To test for differences in the lengths of mountings, we analyzed the mean lengths of mountings for nestmate and non-nestmate pairings using a t-test. Analyses were also performed for subsets of the trials which took into account male activity levels, but these results did not differ from those obtained from analyzing all data, so only the complete results are presented below.

Colony-of-origin

To test for colony-of-origin effects on male and gyne mating success, we counted the number of times males from a particular colony successfully inserted with a gyne. We then calculated expected insertion frequencies for males from each colony based on the number of observed insertions and the number of males from each colony used in all mating trials. The observed number of matings was compared with the expected number of matings for males from the four colonies. Significance was determined using a χ^2 test. The same procedure was performed to analyze colony-of-origin effects on gyne mating success.

Results

Morphometrics

Males from different colonies varied significantly for all nine of the traits measured (ANOVA; HTL, $F_{3, 82}$ = 13.79, p < 0.001; TW, $F_{3, 82}$ = 18.79, p < 0.001; TL, $F_{3, 82}$ = 4.22, p = 0.008; AL, $F_{3, 82}$ = 3.71, p = 0.016; GL, $F_{3, 82}$ = 13.00, p < 0.001; BL, $F_{3, 82}$ = 13.80, p < 0.001; T3L, $F_{3, 82}$ = 10.70, p < 0.001; CL, $F_{3, 82}$ = 10.47, p < 0.001; mass, $F_{3, 82}$ = 17.49, p < 0.001; Table 2.1). This demonstrated that significant variation in male trait

sizes existed between colonies, and that gynes could possibly select mates from different colonies according to trait size.

The values for most pairs of male traits were significantly (α = 0.05) correlated, with the exceptions of TW - TL and TW - T3L (Table 2.2). Correlations between traits were expected, as males with a relatively large value for one trait would also be predicted to show relatively large values for other traits. Principal component analysis of male traits revealed that the first principal component (PC1) accounted for 57% of the total variance, and positively correlated with all measurements. Therefore PC1 was considered to be general body size for males. The second principal component (PC2) accounted for approximately 13% of the total variance and positively correlated with BL, TL, T3L, HTL, AL, and was therefore considered to be length in general.

In a manner similar to the males, gynes were measured and analyzed for variation in morphological traits between colonies. For gynes, only six of the nine traits, TW, TL, HTL, T3L, GL, and BL, showed significant variation between colonies (ANOVA; TW, $F_{4, 51}$ = 4.40, p = 0.004; T3L, $F_{4, 51}$ = 7.62, p < 0.001; BL, $F_{4, 51}$ = 9.23, p < 0.001, GL, $F_{4, 51}$ = 8.02, p < 0.001; HTL, $F_{4, 51}$ = 5.39, p = 0.001; TL, $F_{4, 51}$ = 5.20, p = 0.001; AL, $F_{4, 51}$ = 2.19, p = 0.86; CL, $F_{4, 51}$ = 1.34, p = 0.269, mass, $F_{4, 51}$ = 2.17, p = 0.085; Table 2.1). Surprisingly, many of the traits in gynes were uncorrelated. In total, 14 of the 36 Spearman's correlation coefficients were not significant (Table 2.2). Principal component analysis performed on the gyne traits revealed that PC1 accounted for 48% of the variance. PC1 was positively correlated with all traits and was therefore considered to be general body size. PC2 accounted for 15% of the total variance and was positively correlated with TW, HTL, TL, and AL.

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Table 2.1: Means and standard deviations ($\bar{x} \pm$ SD) of nine size metrics for *V. maculifrons* males and gynes by colony (linear size measurement in mms and mass in mgs). Traits for which means of individuals from different colonies were determined to be significantly different are marked by an asterisk (*). Colony means with different superscript numbers differed significantly ($\alpha = 0.05$) after post hoc tests.

			Colony-of-origin			
	A	B	C	D	E	F
Trait				Males		
Thorax width (TW) *	-	-	$2.73^2 \pm 0.09$	$3.11^1 \pm 0.08$	$2.55^{2,3} \pm 0.08$	$2.33^3 \pm 0.07$
Thorax length (TL) *	-	-	$3.40^2 \pm 0.09$	$3.76^1 \pm 0.07$	$3.78^1 \pm 0.08$	$3.67^{1,2} \pm 0.07$
Head to end of thorax length (HTL) *	-	-	$4.66^3 \pm 0.09$	$5.30^1 \pm 0.07$	$5.00^2 \pm 0.08$	$4.75^{2,3} \pm 0.07$
Tergum 3 length (T3L) *	-	-	$1.54^2 \pm 0.04$	$1.83^1 \pm 0.04$	$1.61^2 \pm 0.04$	$1.61^2 \pm 0.04$
Gaster length (GL) *	-	-	$8.10^{2,3} \pm 0.17$	$8.86^1 \pm 0.14$	$8.27^2 \pm 0.15$	$7.66^3 \pm 0.13$
Antennal length (AL) *	-	-	$7.76^{2,3} \pm 0.13$	$8.00^1 \pm 0.10$	$7.96^{1,2} \pm 0.13$	$7.52^3 \pm 0.12$
Total body length (BL) *	-	-	$13.15^{1,2} \pm 0.23$	$14.59^1 \pm 0.19$	$13.76^{1,2} \pm 0.20$	$13.05^2 \pm 0.18$
Distal cell length (CL) *	-	-	$5.23^{2,3} \pm 0.07$	$5.58^1 \pm 0.06$	$5.38^{1,2} \pm 0.07$	$5.09^3 \pm 0.06$
Mass *	-	-	$19.19^2 \pm 1.12$	$24.27^1 \pm 0.89$	$17.41^2 \pm 0.95$	$16.21^2 \pm 0.85$

Table 2.1 (continued)

	Gynes					
Thorax width (TW) *	$3.67^1 \pm 0.05$	$3.50^{1,2} \pm 0.07$	$3.42^2 \pm 0.06$	$3.62^{1,2} \pm 0.05$	$3.48^2 \pm 0.04$	-
Thorax length (TL) *	$5.32^{1,2} \pm 0.08$	$4.91^{2,3} \pm 0.13$	$5.16^{1,2,3} \pm 0.10$	$5.37^1 \pm 0.08$	$5.00^3 \pm 0.06$	-
Head to end of thorax length (HTL) *	$7.00^1 \pm 0.09$	$6.53^{1,2} \pm 0.14$	$6.75^{1,2} \pm 0.11$	$6.98^1 \pm 0.09$	$6.58^2 \pm 0.07$	-
Tergum 3 length (T3L) *	$2.56^1 \pm 0.06$	$2.18^{2,3} \pm 0.10$	$2.43^{1,2} \pm 0.08$	$2.44^{1,2} \pm 0.07$	$2.17^3 \pm 0.05$	-
Gaster length (GL) *	$8.62^{1,2} \pm 0.24$	$7.52^{2,3} \pm 0.37$	$8.75^{1,2} \pm 0.29$	$8.90^1 \pm 0.25$	$7.51^3 \pm 0.18$	-
Antennal length (AL)	5.17 ± 0.08	5.04 ± 0.11	5.03 ± 0.08	5.22 ± 0.07	4.99 ± 0.05	-
Total body length (BL) *	$16.69^1 \pm 0.25$	$15.20^{2,3} \pm 0.38$	$16.44^{1,2} \pm 0.30$	$16.79^1 \pm 0.26$	$15.30^3 \pm 0.19$	-
Distal cell length (CL)	5.96 ± 0.09	5.63 ± 0.14	5.84 ± 0.11	5.87 ± 0.09	5.76 ± 0.07	-
Mass	53.90 ± 3.82	44.31 ± 6.15	58.58 ± 4.86	61.45 ± 3.97	49.89 ± 3.08	-

Mating success and size

Of the ninety trials conducted, 67 resulted in some mating activity (mounting or insertion). Mounting time of males ranged from a low of 12 seconds to a high of 1830 seconds. In addition, a total of 26 matings were observed in fifteen different trials. Few male-male interactions were observed during the trials; in a few cases, a male would attempt to mount a male that was either mounting the gyne or had just ceased mounting the gyne. Gynes were seen biting males both during and immediately following mountings, indicating that gynes were not passive participants in the mating process. Most mountings ended when the male fell off or was knocked off by the gyne after unsuccessfully attempting to insert.

No significant correlations were found between the size of male traits and mating success. In the subset of fifteen trials in which insertions were observed, there was no significant difference in mating success between males possessing smaller or larger traits (Fig. 2.2 A; sign test, p > 0.05; power analyses were run for data subsets; for a subset of 15 trials and α = 0.05, a result of n = 3 trials would be statistically significant). In the subset of 61 trials in which mating activity (mounting or inserting) differed between the paired males, no effect of trait size was detected (Fig. 2.2 B; sign test, p > 0.05; power analysis, α = 0.05, n = 22). In the subset of thirty-seven trials where only one male exhibited mounting behavior, the male that procured all mountings did not possess larger or smaller traits at a significantly different frequency than the male that exhibited no activity during the trial (Fig. 2.2 C; sign test, p > 0.05; power analysis, α = 0.05, n = 12).

Next, we investigated whether any gyne traits were associated with mating success. We found that gynes with longer gasters, third terga, total body lengths, and antennal lengths mated at a higher frequency than those with smaller traits (Fig. 2.3; GL,

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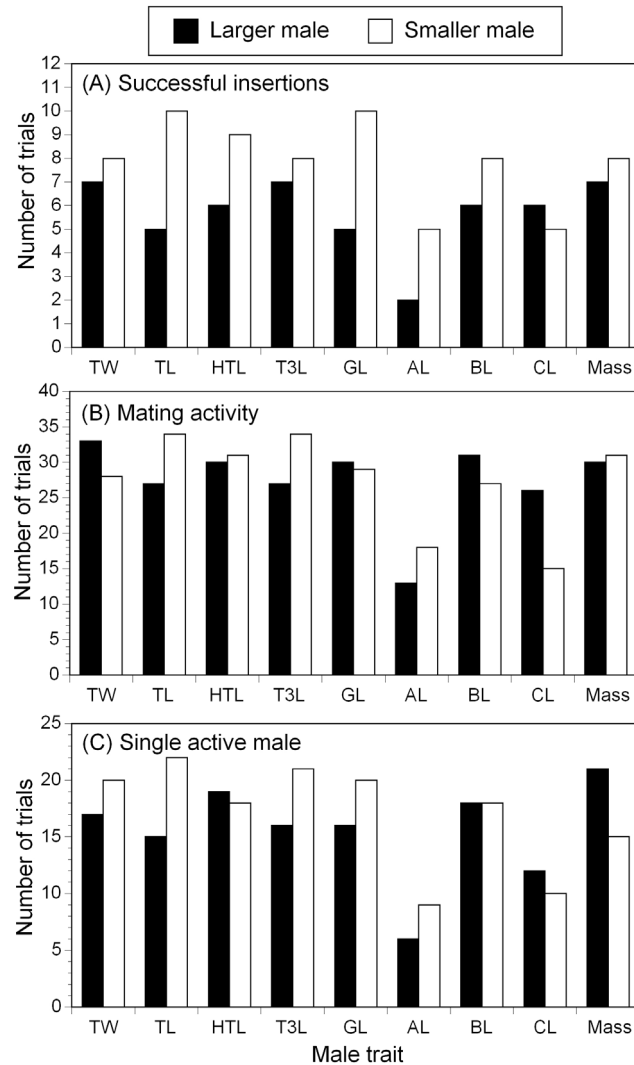
Figure 2.2: Relationship between male mating behavior and male trait size in the social wasp *V. maculifrons*. "Larger male" and "Smaller male" bars indicate the number of times the male with the larger and smaller trait exhibited more mating behavior, respectively. (A) "Successful insertions" panel only includes trials in which a successful insertion was observed. (B) "Mating activity" panel only includes trials in which at least one male was observed mounting the gyne. (C) "Single active male" panel only includes trials in which one male was observed mounting the gyne. Overall, no significant effects of trait size on male mating activity were observed. (See Table 1 for trait abbreviations.)
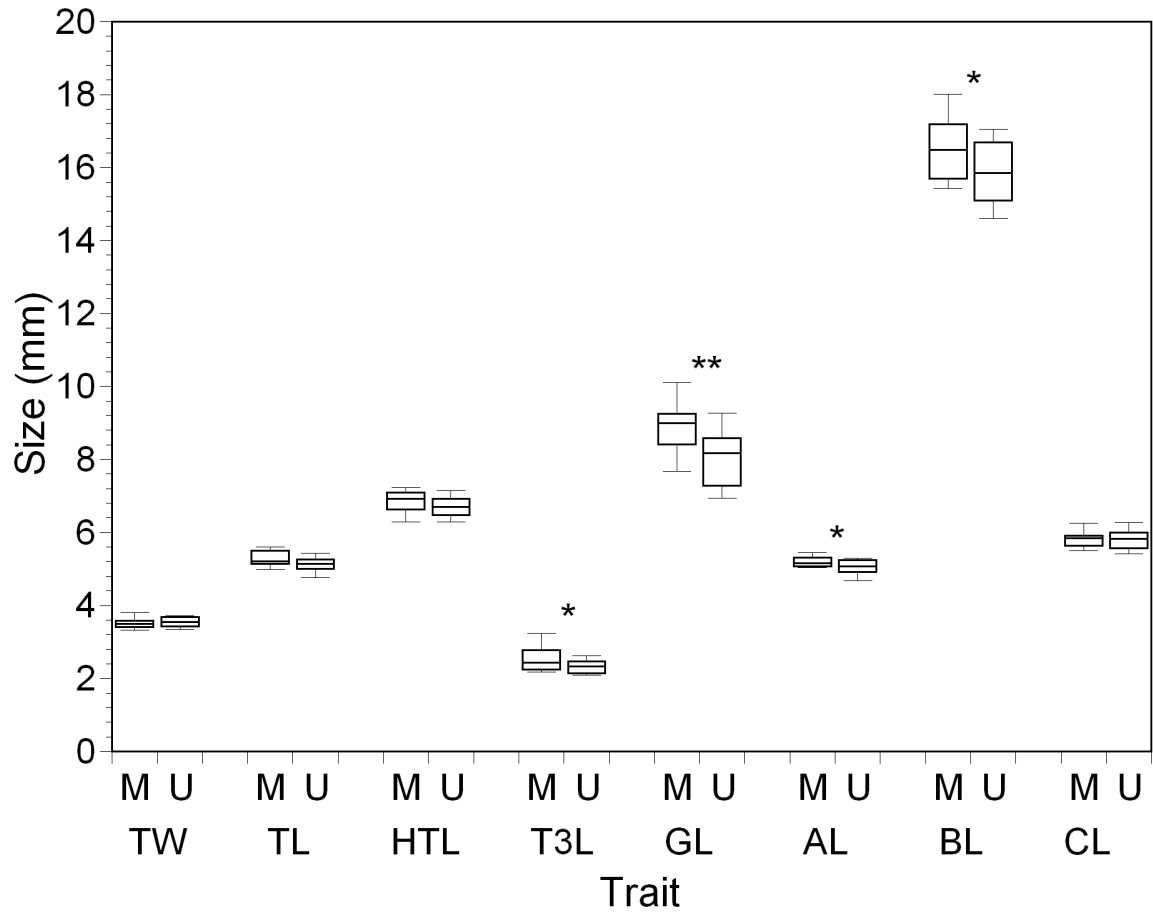
Figure 2.3: Box plots illustrating the size of linearly measured traits in mated (M) and unmated (U) *V. maculifrons* gynes. Mated gynes were significantly (* $p < 0.05$; ** $p < 0.01$) larger than unmated gynes for four traits: T3L, GL, AL, and BL (See Table 1 for trait abbreviations).

Table 2.2: Spearman's correlation coefficients (r_s) between size metrics for *V. maculifrons* gynes (above the diagonal) and males (below the diagonal). Statistically significant correlations are shown in bold (α = 0.05). See Table 1 for explanation of abbreviations.

Trait	TW	TL	HTL	T3L	GL	AL	BL	CL	Mass
TW	-	**0.432**	**0.462**	0.243	0.224	**0.560**	**0.359**	0.070	0.134
TL	0.026	-	**0.809**	**0.494**	**0.546**	**0.498**	**0.701**	0.203	0.332
HTL	**0.400**	**0.674**	-	**0.341**	**0.445**	**0.498**	**0.694**	0.096	0.210
T3L	0.146	**0.376**	**0.421**	-	**0.711**	0.383	**0.616**	0.198	**0.440**
GL	**0.572**	**0.372**	**0.640**	**0.414**	-	**0.480**	**0.880**	0.071	**0.488**
AL	**0.377**	**0.445**	**0.563**	**0.364**	**0.578**	-	**0.507**	0.285	0.317
BL	**0.460**	**0.514**	**0.826**	**0.511**	**0.894**	**0.679**	-	0.127	**0.468**
CL	**0.544**	**0.323**	**0.540**	**0.335**	**0.565**	**0.602**	**0.588**	-	**0.349**
Mass	**0.596**	0.245	**0.545**	**0.364**	**0.742**	**0.475**	**0.703**	**0.619**	-

t_{78} = -3.171, p = 0.002; T3L, t_{78} = -2.436, p = 0.017; BL, t_{78} = -2.294, p = 0.025; AL, t_{60} = -2.006, p = 0.049). Size in other traits was not associated with mating success for gynes Fig. 2.3; TW, t_{78} = 0.339, p = 0.736, TL, t_{74} = -1.571, p = 0.121, p = 0.446; HTL, t_{78} = -1.092, p = 0.278; CL, t_{80} = -0.174, p = 0.863; mass, t_{82} = 0.765). The number of significant results observed was greater than expected by chance alone and the data thus suggest that long gynes had higher mating success than short gynes.

Inbreeding avoidance

Of the 91 total mountings observed, 43 were with nestmates and 48 with non-nestmates (sign test, p > 0.05). Thus, there was no significant difference in the numbers of mountings procured by nestmate versus non-nestmate males. The mean length of a mounting for nestmates was 78.37 seconds, and for non-nestmates 63.13 seconds; these means are not significantly different (two-tailed t-test, t_{36} = 0.852, p = 0.401).

Colony-of-origin

We found a strong association between the number of insertions a male obtained and the colony from which he originated. Specifically, males from colony C showed a higher than expected number of matings (Fig. 2.4 A; males: χ^2_3 = 34.2, p < 0.001). These males also showed a significantly greater amount of mounting behavior than males from other colonies (ANOVA, $F_{3, 176}$ = 12.75, p < 0.001). In addition, we found that gynes from colony C showed a higher than expected number of matings given the number of trials in which they participated (Fig. 2.4 B; gynes: χ^2_4 = 46.6, p < 0.001).

Discussion

The goal of this study was to investigate mating behavior and mating success in
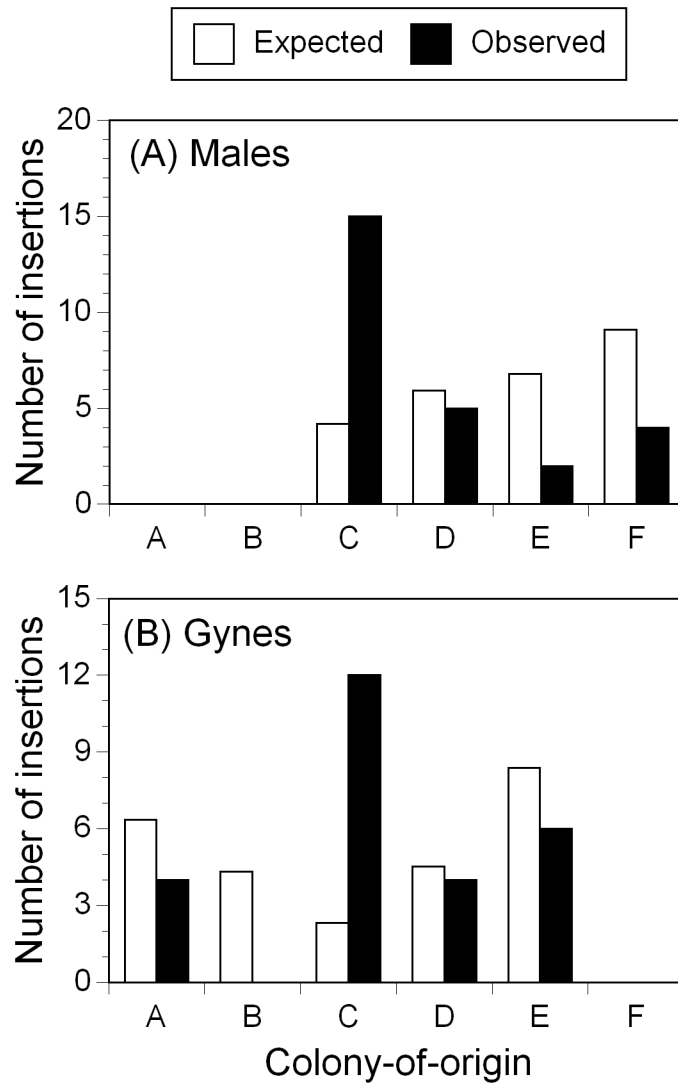
Figure 2.4: Expected and observed numbers of insertions for *V. maculifrons* males and gynes from six colonies (A-F). There were significant differences in the expected and observed number of insertions for both sexes (p < 0.001; see text for details). In particular, males and gynes from colony C mated more often than expected.

the polyandrous social wasp *V. maculifrons*. We wished to test our theory suggesting that trait size would be associated with mating success for males and gynes (pre-reproductive females). In addition, we were interested in determining if relatedness and colony-of-origin influenced mating behavior. We found that males showed significant variation in all trait sizes between colonies.

However, trait size was not associated with male mating success. In contrast, gynes exhibited size variation for fewer traits than males, and longer gynes mated at significantly higher rates than shorter gynes. Additionally, we discovered that males and gynes from one particular colony exhibited higher than expected mating and mounting success.

Variation in size of gynes and males

Males from different colonies differed significantly for all nine measured traits. In addition, gynes from different colonies differed significantly for six of the nine measured traits. Thus, our data suggest that either the genetic or environmental variation that distinguishes colonies leads to variation in the size of sexuals among colonies.

The greater variability of males than gynes among colonies may be a product of the way in which the two sexes are reared in this species. Gynes are always reared in specialized queen cells that are constructed toward the end of the colony cycle and dedicated solely to the rearing of gynes. Thus gynes may be somewhat buffered from displaying substantial variation in the size of certain traits. In contrast, males can develop in either worker comb surrounded by developing workers or in queen comb where they are surrounded by developing gynes (EDWARDS 1980). The variation in male size may thus be a product of differential worker feeding of larvae in different types of comb. Gynes may receive greater worker attention, be reared under more uniform conditions, and therefore achieve consistently large body sizes. In contrast, male size

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may be greatly affected by environmental fluctuations in food availability and the diverting of food to gynes later in the season, resulting in significant amounts of size variation among males.

Variation in the size of sexuals between colonies has been documented in other social insect species. In the ant *Pogonomyrmex occidentalis*, male size varies between colonies and is thought to be the product of differential worker investment due to environmental conditions (WIERNASZ *et al.* 2001). Intrasexual differences in both gyne and male body size between colonies have also been documented in the ant *Lasius niger*. In this species both environmental and genetic factors are thought to influence variation in sexual body size (FJERDINGSTAD 2005). However, both female and male sexuals are reared alongside worker brood in ants. This may expose developing ant sexuals to substantial environmentally variable feeding. In contrast, wasp sexuals, which are reared in specialized comb, may not be subject to such large amounts of environmental variation.

Size variation could be significant if it leads to higher mating success (see following sections) or to higher viability. For example, gyne size may be under strong selection if large gynes have a higher probability of surviving mating flights and overwintering than small gynes. Large gynes may also be more successful at establishing new colonies. Thus, larger gynes may enjoy a viability advantage relative to small gynes. In contrast, males, which survive only long enough to mate, may not be under strong selective pressure for large body size if male-male competition is uncommon or does not involve physical interactions, as is thought to be the case in *V. maculifrons* (SPRADBERY 1973). Therefore, selection may not act as strongly on male size as it does on female size in this taxon.

Male trait size and mating success

Though this study found significant variation in male trait size among colonies, no associations between the size of male traits and male mating success were detected (Fig. 2.2). The lack of association between male trait size and mating success suggests that males are not selected by gynes for large size or that male size does not affect male-male competition. However, it is possible that male size may not be a reliable indicator of male fitness due to the strong influence that environment may play in determining male size.

The effect of male size on mating success has been examined in several species of social insect (ABELL *et al.* 1999; WIERNASZ *et al.* 1995; OBERSTADT & HEINZE 2003). In the lekking ant species *Pogonomyrmex occidentalis*, male size is a strong indicator of male mating success (WIERNASZ *et al.* 1995). Though no one trait was found to determine mating success in *P. occidentalis*, the shape of some traits such as the mandibles may have played a part in mate choice in females of that species, and may indicate a male's physical ability to secure mates (ABELL *et al.* 1999). In contrast, no effect of male size on mating success has been reported for the ant species *Leptothorax gredleri* (OBERSTADT & HEINZE 2003). Though trait sizes between males that did mate and those that did not mate were not specifically examined in that study, no difference in the size of males who mated multiply and those that mated singly were discovered. Thus, it appears that male size may not be as relevant in determining mating success in social insects as it is in other insect and vertebrate taxa (ANDERSSON 1994; BLANCKENHORN 2005).

Gyne trait size and mating success

We found that mating success for gynes was positively correlated with gyne gaster length, antennal length, tergum 3 length, and total body length (Fig. 2.3). Thus, longer gynes had a higher probability of mating than shorter gynes. We suggest two

possible explanations for this finding. The first explanation invokes male mate choice based on gyne viability. Gyne size has been positively correlated with overwintering survival and fecundity in other social insect species (WIERNASZ & COLE 2003; FJERDINGSTAD & KELLER 2004). If this is also the case in *V. maculifrons*, and males are sperm limited, then males may practice mate choice and preferentially mate with the longer gynes, which are likely to ultimately produce more offspring than shorter gynes. However, our experiments did not specifically allow us to test this hypothesis, because males were not given a choice of gynes during mating trials. A second explanation for the observed correlation between gyne mating success and gyne length is that longer gynes may be more capable of successfully undertaking mating behaviors and may be more receptive to mating. Thus increased mating competence alone may explain the increased mating frequency of longer gynes.

FJERDINGSTAD & KELLER (2004) detected an effect of gyne phenotype on mating success based on mating frequency in the ant *Lasius niger*. Gynes with greater body mass were found to have mated at a higher frequency than those with smaller body masses. Larger body mass was also found to be significantly correlated with mating flight survival, the number of brood present at the time of first eclosion, and the colony productivity during one year of the study. These findings may indicate that female size is an important factor in female mating success in social insects due to its tight correlation with female fitness.

Inbreeding avoidance

We did not detect evidence of nestmate avoidance during our mating trials. A lack of nestmate avoidance in laboratory settings has previously been reported in this species (ROSS 1983) as well as other species of social insects both in the laboratory and the field (COLE & WIERNASZ 1997; KELLER & FOURNIER 2002). These previous studies

suggested that wide dispersal and nuptial flights prior to mating activity may act as a satisfactory method of nestmate avoidance and thereby preclude the need for the evolution or the maintenance of mechanisms for inbreeding avoidance or nestmate recognition. ROSS (1983) reported mating activity being inhibited by darkness in *V. maculifrons*, thereby providing a behavioral mechanism by which in-nest inbreeding is avoided. Indeed genetic studies have demonstrated a lack of evidence for frequent inbreeding in natural populations of vespine wasps (GOODISMAN *et al.* 2001).

Colony-of-origin

Both males and gynes from Colony C mated significantly more often than expected (Fig. 2.4). Males from Colony C also mounted significantly more than males from other colonies. One mechanism that could explain this finding is that males from Colony C were more active than males from other colonies and therefore procured more matings. The males from all colonies were treated equally, so there is no evidence that this increased activity arose through an artifact of laboratory conditions. Rather, the high sexual activity exhibited by individuals from this colony may result from genotype or colony environment.

We note that long gynes were found to mate at higher frequencies than short gynes (see above). However, gynes from Colony C did not have the longest traits, though they did not possess the shortest traits either (Table 2.1). In addition, when Colony C gynes were removed from the analysis, gynes possessing long traits were still found to mate at significantly higher frequencies than short gynes, though total body length was no longer significant (t-test, gaster length, t_{70} = -2.92, p = 0.005; tergum 3, t_{70} = -2.57, p = 0.012; mean antennal length, t_{52} = -2.36, p = 0.022; total length, t_{70} = -1.71 p = 0.093). Thus, it seems that both gyne length and colony-of-origin have significant effects on the mating success of gynes.

Recent studies in *Bombus* have revealed a possible effect of both male and queen colony-of-origin on queen overwintering and survivorship. BAER & SCHMID-HEMPEL (2005) found that queen longevity depended on which male's sperm was used to inseminate the female. Another study discovered that *Bombus* queens from certain matrilines had higher rates of hibernation survival and colony foundation success (GERLOFF & SCHMID-HEMPEL 2005). These results, in addition to our findings, indicate that colony-of-origin may be a factor in mate choice due to possible long-term benefits such as hibernation survival, queen longevity, and higher colony fitness. Further work will help us better understand the mechanisms and effects of colony-of-origin on mating success and the possible long-term benefits associated with colony-of-origin.

Acknowledgements

CHAPTER 3[2]

I WILL SURVIVE: BODY LENGTH IS ASSOCIATED WITH FEMALE FITNESS IN A

SOCIAL WASP

Abstract

Body size can play an important role in determining an individual's fitness.

Female body size is positively associated with survival and fecundity in a number of

animal species. In this study, we identified female morphological traits associated with

two fitness related events, overwintering and mating, in the social wasp *Vespula

maculifrons*. Longer females survived overwintering for a greater duration than shorter

females in multiple study years. Female length was also associated with mating

success, though the association was not positive in all years. In addition, we uncovered

a genetic component to female mating success. Overall, these findings suggest that

female length is an important determinant of female fitness, and therefore may be under

natural, as well as sexual selection in this species.

Introduction

Body size is a large component of an individual's phenotype and can have major

effects on individual reproduction, mating success, dispersal, and survival. For example,

large male size is often positively correlated with reproductive success due to its

importance in securing both resources and mates (ANDERSSON 1994). Additionally,

female body size has also been shown to play a role in determining female reproductive

fitness. Though attaining large body size may carry fitness costs due to trade-offs

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between somatic growth and reproduction (ROFF, 1992), large female size is often

directly associated with high fecundity in many animal species (reviewed by

BLANCKENHORN 2005).

Among social insects (ants, termites, some bees, and some wasps), reproductive

female (queen) size has been positively linked to survival and reproduction. Among ants

with multiple colony-founding strategies, queens that found colonies independently are

significantly larger, have more fat reserves, and disperse further than queens that found

new colonies with other queens, return to their maternal colony, or join already

established nests (HEINZE 1989; KELLER & PASSERA 1989; ELMES 1991; SUNDSTRÖM

1995; RÜPPELL *et al.* 1998; RÜPPELL & HEINZE 1999; PEETERS & ITO 2001). Additionally,

large body size can increase a queen's ability to survive mating flights and colony

founding, as well as maintain a colony and workforce through to the production of new

sexuals (WIERNASZ & COLE 2003; FJERDINGSTAD & KELLER 2004). Therefore, a queen's

reproductive fitness is strongly dependent upon her ability to survive long enough to

produce sexuals.

Our primary interest in this study was to explore factors affecting female fitness in

the social wasp *Vespula maculifrons*. We were particularly interested in morphological

traits associated with two fitness events, overwintering survival and mating success.

Overwintering, the one to three month period subsequent to mating during which

Vespula queens hibernate, results in high levels of mortality (ROSS 1983). Therefore,

queen traits associated with overwintering survival are expected to also be associated

with female fitness. If queen survival is influenced by queen morphological trait size,

then those traits would be subject to natural selection (WIERNASZ & COLE 2003).

Additionally, we determined whether the same morphological traits influencing

overwintering survival were also associated with queen mating success. Female

morphological trait sizes, particularly length and weight, have been identified by previous

studies as being positively correlated with increased mating success as well as fecundity

(reviewed by BONDURIANSKY 2001). Therefore, queen morphological traits influencing

queen mating success would also serve as indicators of female fitness due to their

association with increased fecundity. If queen size is associated with overall female

fitness, then males could use size as a cue of female fitness. Males are predicted to

exhibit mating preferences in mating systems in which there is a high variance in female

fitness (other factors involved in the evolution of male mate choice are discussed below;

BONDURIANSKY 2001). In social insects, a male's reproductive fitness is dependent upon

his mate's ability to survive through to the production of sexuals. As discussed above,

social insect queens face high mortality throughout their life-histories, and queen size is

expected to play a large role in determining survival. Therefore, if male mate choice is

operating in this species, we would expect males to mate with larger queens because

such queens would have a higher probability of surviving long enough to produce

sexuals. Thus, this study addresses two questions: 1) is queen size associated with

overwintering survival and 2) does queen body size influence female mating success.

Queen morphological traits associated with both overwintering survival and queen

mating success could therefore be under natural and/or sexual selection due to their

influence on female fitness.

Our secondary interest in this study was to determine if genetic factors were

associated with overwintering survival and queen mating success. Because *V.*

maculifrons queens mate with many males, their offspring possess a substantial amount

of genetic diversity (ROSS 1983; GOODISMAN *et al.* 2007b). Producing genetically diverse

offspring may be beneficial if genetic incompatibilities between males and females result

in decreased reproductive fitness (ZEH & ZEH 1997). Also by producing a genetically

diverse cohort of daughters, a queen may increase the probability that some of her

daughters survive to reproduction if environments are variable and certain genotypes are

favored under different environmental conditions (CROZIER & PAGE 1985). Thus, the finding of variation in fitness components among individuals with different genotypes would provide opportunity for selection and may support the hypothesis that multiple mating by *V. maculifrons* queens is adaptive (CROZIER & FJERDINGSTAD 2001).

Methods

Sample Collection

Mature *V. maculifrons* colonies containing males and pre-reproductive queens (gynes) were collected from metropolitan Atlanta, GA, USA during the autumn of 2004 (N = 5 colonies), 2006 (N = 8 colonies), and 2007 (N = 2 colonies). Males and gynes were separated from each other and the rest of the colony in the laboratory.

Mating Trials

Much of what is known about *V. maculifrons* mating behavior comes from in-lab mating experiments (ROSS 1983; KOVACS *et al.* 2008) and anecdotal observations (SPRADBERY 1973). From these sources we know that mating in *V. maculifrons* occurs when gynes are on substrates rather than in flight (SPRADBERY 1973) and has been described as occurring in several distinct stages (ROSS 1983). First, a male orients towards a gyne when he is 5-15 cm away from her. The male then approaches and mounts the gyne. He then grips her and attempts to insert his reproductive organs into her sting chamber. At this point, the gyne often appears to try to repulse the male's mating attempt by twisting or pushing him off. Nevertheless, males do successfully mate with gynes, thereby transferring sperm. Little is known about the role of pheromones in *V. maculifrons* mating behavior, though observations of male-male mounting behavior subsequent to male mating are suggestive of chemoattraction (ROSS 1983; KOVACS

personal observation). Additionally, mate choice has not been previously examined in this species.

To identify gyne morphological traits associated with mating success, we conducted mating trials under laboratory conditions during the three study years. Males and gynes were marked and numbered so that individuals could be easily identified throughout mating and overwintering trials. The design of the 2004 mating trials as well as the data collected from that year were originally reported in KOVACS *et al.* (2008). Briefly, in 2004 single gynes were placed in mating arenas with combinations of large and small and nestmate and non-nestmate males; trials were observed continuously for 45 minutes. In 2006, mating trials were conducted with single gynes and 3 to 5 non-nestmate males in each mating arena. In 2007, mating arenas contained multiple gynes (2-4 gynes) and multiple non-nestmate males (4-7 males). In 2006 and 2007, mating trials were continuously observed for 45 minutes or until a male successfully mated with a gyne. In all years, males and gynes were used in multiple trials, and gynes with which a male had successfully inserted for 45 seconds or longer were considered mated. In total over the three study years, 97 successful copulations (2004 n = 26, 4 gynes mated multiple times ranging from 2 to 5 times within the same trial; 2006 n = 35, 3 gynes were mated twice during separate trials; 2007 n = 36, 4 gynes were mated twice during separate trials) were observed during a total of 1021 observed mating trials (2004 n = 90; 2006 n = 500; 2007 n = 431).

Overwintering Trials

To determine which gyne morphological traits were associated with overwintering survival, overwintering trials were performed using a method modified from ROSS *et al.* (1981). Gynes were placed in ventilated containers. Each container was filled with

moistened paper towels and stored at 4º C. Once a week, the paper towels were changed and dead gynes were removed for further analyses.

Gynes used in mating trials in 2006 and 2007 were overwintered (mated gynes: 2006: n = 15; 2007: n = 32; unmated gynes: 2006: n = 89; 2007: n = 126). In addition, unmated gynes that had not been used in mating trials were also overwintered in 2006 (n = 55). However, gynes from 2004 were not overwintered.

Morphological Measurements

Longer *V. maculifrons* gynes have been found to mate at higher frequencies than shorter gynes (KOVACS *et al.* 2008), suggesting that gyne size is an important fitness-related trait. Therefore, in this study, we attempted to determine if gyne body size was associated with both gyne mating success and overwintering survival. We first recorded the size of several morphological traits for gynes used in 2004 mating trials, as well as 2006 and 2007 mating and overwintering trials. Gynes removed from overwintering trials were photographed in a dorsally facing position. A Zeiss© millimeter micrometer (Carl Zeiss Microimaging, Thornwood, NY, USA) was included in each photo to allow for size calibration. Images were analyzed using tools within the Canvas 9.0.4 computer package (ACD Systems, Victoria, BC, Canada) to obtain measurements of thorax width (TW), thorax length (TL), head to thorax length (HTL), third tergum length (3TL), gaster length (GL; the measure of the abdomen not including the first abdominal tergum), and total body length (BL).

Genetic Analysis

Vespula maculifrons queens exhibit high levels of polyandry and typically mate with between three to nine males (ROSS 1983; GOODISMAN *et al.* 2007a; 2007b). Polyandry results in multiple patrilines being present among cohabitating individuals

within a colony. Subsequent to mating and overwintering trials, we assigned gynes to patrilines within colonies using genetic techniques. Specifically, DNA from gynes for all three years was extracted from leg samples using modifications of the Chelex extraction (WALSH *et al.* 1991). DNA was then amplified at five highly variable microsatellite markers (GOODISMAN *et al.* 2007b) and run on an ABI 3100 sequencer. Alleles were visualized using the ABI program Genemapper™. Gynes were then assigned to patrilines within colonies based on their multilocus genotype as described by HOFFMAN *et al.* (2008).

In 2007, we genotyped 155 gynes from two colonies (unmated: $n = 124$, colony A: $n = 60$; colony B: $n = 55$; mated: $n = 31$, colony A: $n = 25$, colony B: $n = 6$) We were unable to amplify the DNA for one mated gyne from colony A at enough loci to assign her unambiguously to a patriline.

Statistical Analyses

To gain a preliminary understanding of the effects of gyne size on overwintering success, linear regression was used to gain initial information on whether there was a correlation between the week a gyne died during the overwintering trials and her trait size. Similarly, to determine gyne morphological traits that were associated with mating success, we used ANOVA to determine if individual trait sizes of gynes that successfully mated differed from those that failed to mate (whether a gyne mated or not is hereafter referred to as gyne 'matedness' or 'mating success'). Additionally, due to the differences in the mating trials in different years, univariate analyses were performed separately by year to allow for the evaluation of differences between years.These univariate analyses provided initial assessments of the effects of trait size on overwintering survival and mating success. However, covariation among traits may give misleading results about the contribution of individual traits to fitness (LANDE & ARNOLD 1983). We therefore we

employed multivariate techniques to accurately dissect the influence of morphology on overwintering survival and mating success.

Prior to multivariate regression analyses (e.g., JANZEN & STERN 1998), size measurements were standardized to mean of zero and unit variance within each year. Due to complications in multivariate analyses that can arise due to high correlations between traits (LANDE & ARNOLD 1983, SCHLUTER & SMITH 1986), phenotypic correlations between traits were first estimated using Pearson's correlations. We then selected the subset of morphological traits that were relatively uncorrelated for use in our analyses of overwintering survival and mating success (hereafter referred to as uncorrelated traits = TW, TL, 3TL, and GL). The two morphological traits (HTL and BL) that were highly correlated with at least one other trait ($r^2 > 0.5$) were dropped from the model. In order to detect the influence of morphological traits on overwintering survival, we used least square regressions performed on the standardized subset of uncorrelated morphological traits. We included year and colony nested within year as independent variables in these analyses to ascertain if gynes from different colonies or years differed in survivorship. We also included colony and patriline nested within colony as variables in our analysis of the 2007 data to determine the effect of genotype on overwintering survival. We then used ANOVA to assess whether the length of overwintering survival differed between mated and unmated gynes.

We then employed multivariate analyses to determine which factors affected gyne mating success. We used the standardized subset of uncorrelated traits as independent variables and mating status as the dependent variable in a generalized linear model (binomial distribution, logit linked). We were also interested in determining if gynes sired by different males within colonies differed in mating success. We used exact tests on the 2007 data as implemented by the program STRUC from the GENEPOP software package (RAYMOND & ROUSSET 1995) to determine if the number of mated and

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unmated gynes differed among patrilines within colonies (low numbers of gynes belonging to different patrilines in 2004 and 2006 precluded using these samples in our analyses). The resulting *P*-values for each colony were then combined using a Z-transform test (WHITLOCK 2005). Additionally, we performed multivariate analyses including colony and patriline nested within colony as independent variables in our model to determine if patriline was associated with mating success.

Additionally, we employed generalized linear models to determine the effects that principal components (PCs) derived from gyne size measurements had on survival (standard least square regression) and mating (generalized linear model: binomial distribution, logit linked; LANDE & ARNOLD 1983; BRODIE *et al*. 1995). The results obtained using the PC generalized linear models were consistent with those returned by the multivariate regression analyses and therefore are not reported.

Results

Factors Influencing Overwintering Survival

The primary aim of this study was to determine traits that were associated with overwintering survival and gyne mating success, two fitness events that would provide a measure of female quality. We began by identifying morphological traits associated with overwintering success using univariate analyses. Using a bivariate analysis of fit, we found that two traits, GL and BL, were positively correlated with overwintering survival in the two years that
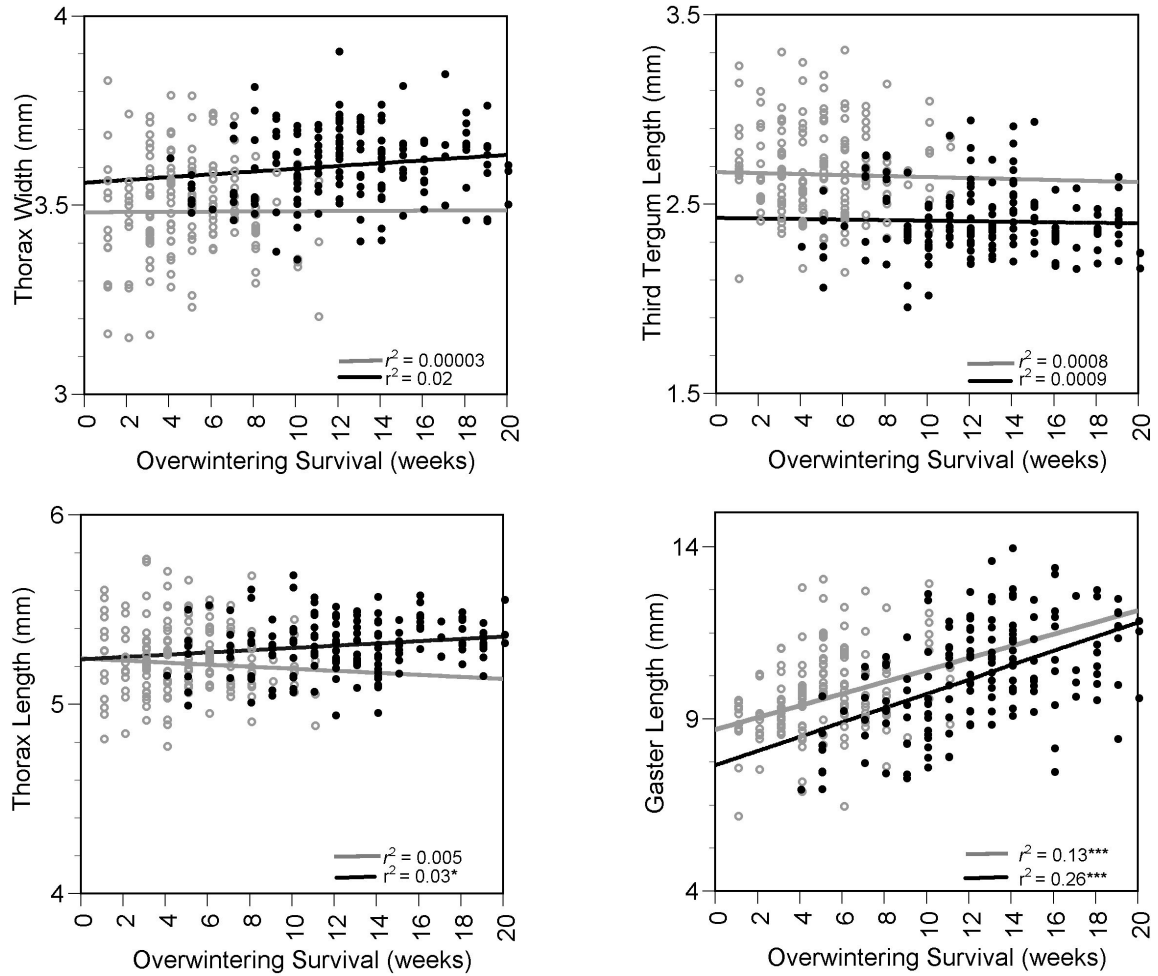
Figure 3.1: Association between trait size and overwintering survival. The size of gyne thorax length in one year and gaster length in both years were significantly and positively associated with overwintering survival (* *P* < 0.05, *** P < 0.001). Gray and black symbols represent data from 2006 and 2007, respectively.

Table 3.1: Results of multivariate least squares regressions testing for the effects of trait size, year, colony within year, and patriline within colony on the overwintering survival of *V. maculifrons* gynes. In 2006 & 2007 year, colony (year), and gaster length all had a significant effect on overwintering survival. In 2007, both gaster length and colony significantly affected overwintering survival.

Variable	2006 & 2007 combined	2007
Year	$F_{1, 303} = 618.01$***	--
Colony(year)	$F_{8, 303} = 4.65$***	--
Thorax Width	$F_{1, 303} = 0.29$	$F_{1, 139} = 0.91$
Thorax Length	$F_{1, 303} = 1.84$	$F_{1, 139} = 1.11$
Third Tergum Length	$F_{1, 303} = 3.00$	$F_{1, 139} = 2.35$
Gaster Length	$F_{1, 303} = 89.53$***	$F_{1, 139} = 52.14$***
Colony	--	$F_{1, 139} = 4.92$*
Patriline(colony)	--	$F_{10, 139} = 1.57$

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$

overwintering trials were conducted (2006 and 2007). Additionally HTL and TL were significantly

and positively associated with overwintering survival in one year, but not the other (Fig. 3.1;

data shown only for uncorrelated traits; see above). The multivariate analyses supported the

findings of the univariate analyses. GL was highly significant in the generalized linear model

performed on the subset of uncorrelated traits (Table 3.1). Thus our results demonstrate that

longer gynes, particularly those with longer gasters, survived better than shorter gynes in

overwintering trials.

Furthermore, we determined whether colony and patriline affected overwintering

success in order to understand the importance of genetic effects on gyne survival. Our analyses

revealed that colony strongly influenced overwintering survival, while patriline did not (Table

3.1). Moreover, in the generalized linear models, colony and GL were highly significant within

the model, while patriline was not (Table 3.1). Thus our data suggested that both colony and

trait size strongly influenced overwintering survival, but gyne genotype did not.

Finally, we tested if mated gynes survived overwintering longer than unmated gynes. However,

we found no significant evidence that mated and unmated gynes survived significantly different

lengths of time (ANOVA: 2006, $F_{1,103}$ = 0.67, P = 0.414; 2007, $F_{1,155}$ = 0.03, P = 0.870). Thus

matedness did not impact overwintering survival in *V. maculifrons*.

Factors Influencing Mating Success

We next turned our attention to determining which traits were associated with gyne

mating success, and whether these traits were also associated with overwintering survival, as

would be expected if these traits were determinants of female quality in this species. We began

by identifying morphological traits associated with mating success using univariate analyses.

Mated and unmated gynes differed significantly in the sizes of three traits, GL, 3TL, and BL

though, surprisingly, not in a consistent direction (Fig. 3.2; data shown for uncorrelated traits

only; see above). In particular, the factors affecting mating success were similar in 2004 and 2007. However, a different set of factors affected mating success in 2006.

In 2004 and 2007, mated gynes had significantly longer GLs and BLs than unmated gynes. Additionally, mated gynes had longer 3TLs than unmated gynes in 2004. Thus, our univariate analyses suggested that longer gynes were more likely to be mated in 2004 and 2007. These results were supported by our multivariate analyses. The generalized linear models performed using a subset of four uncorrelated standardized traits revealed that GL explained mating success in both years (Table 3.2). Thus the multivariate analyses indicated that gynes with longer gasters enjoyed higher mating success in 2004 and 2007.

In contrast, mated gynes had significantly *shorter* GL and BL than unmated gynes in 2006 (GL; Fig. 3.2). The generalized linear models performed using the subset of four uncorrelated standardized traits also revealed that GL was a significant explanatory variable for mating success in 2006 (Table 3.2). Consequently, the multivariate analyses indicated that shorter gynes mated at higher frequencies in 2006, in opposition to the results from 2004 and 2007. Thus, overall, several morphological traits, particularly those associated with length, differed significantly between mated and unmated gynes each year, but the direction of the association was dependent on year.

We next turned our attention to the question of whether colony or patriline influenced gyne mating success. We found a significant effect of patriline on mating status in that year with gynes from some patrilines mating significantly more often than those from others (Z-transformed exact test: $P = 0.001$; Fig. 3.3). Additionally, the generalized linear model, which used uncorrelated morphological traits as explanatory variables, uncovered a significant effect of patriline on mating status (Table 3.2). Thus gyne genotype influenced mating success in our mating trials.
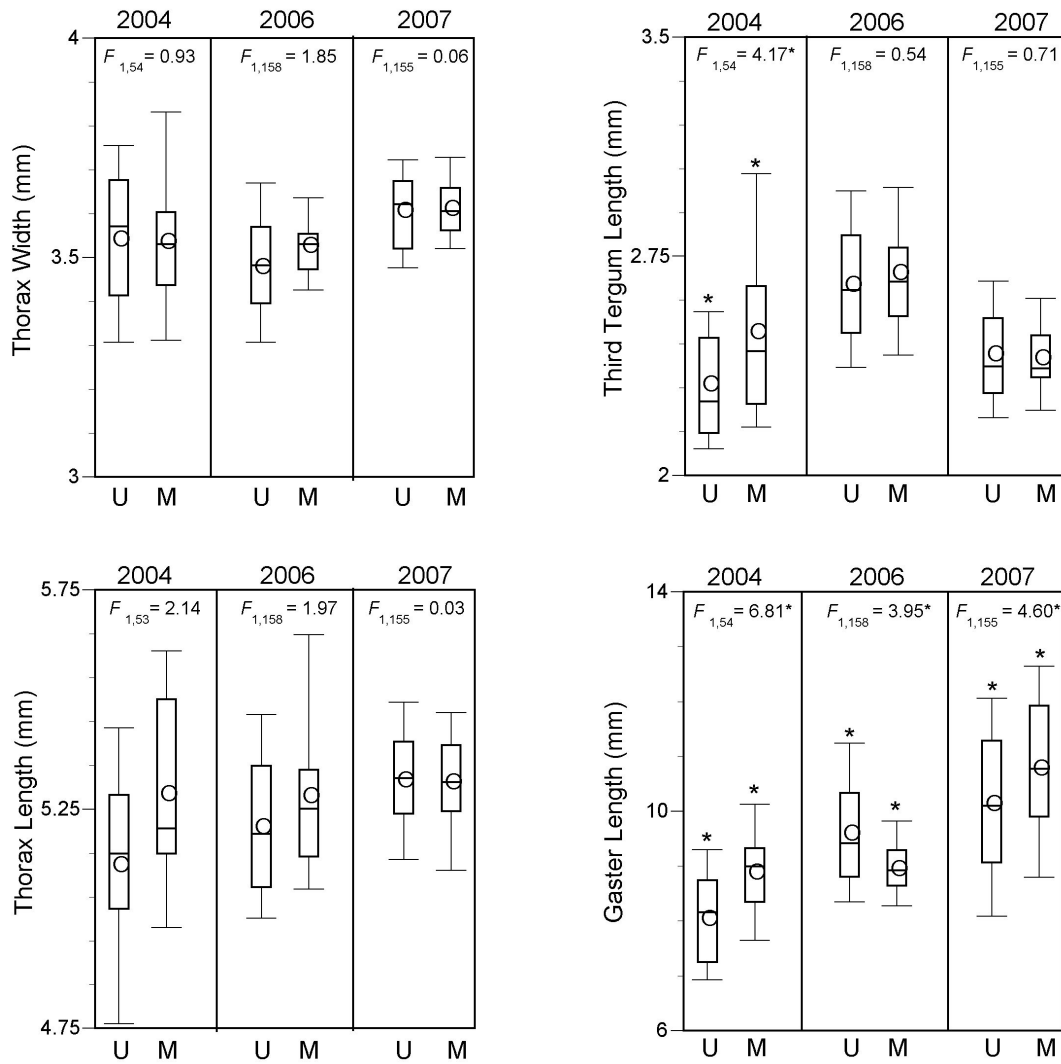
Figure 3.2: Box plots displaying sizes of uncorrelated traits in mated (M) and unmated (U) *V. maculifrons* gynes (* denotes significant differences in size at a threshold of *P* < 0.05). Mated and unmated gynes differed significantly in their mean gaster lengths in all three study years, though not in consistent directions. The top, middle, and bottom lines in each box correspond to the 75th, 50th, and 25th percentiles, respectively. The whiskers represent the 10th and 90th percentiles, and the circles denote the mean.

Table 3.2: Results of generalized linear models (binomial distribution, logit linked) testing for the effects of sizes of four morphological traits, colony, and patriline on the mating success of *V. maculifrons* gynes. Gaster length was significantly associated with gyne mating success in all three years. In 2007 both gaster length and patriline were significantly associated with mating success.

Year	Thorax Width (χ^2_1)	Thorax Length (χ^2_1)	Third Tergum Length (χ^2_1)	Gaster Length (χ^2_1)	Colony (χ^2_1)	Patriline (χ^2_{10})
2004	0.30	0.56	0.11	3.84*	--	--
2006	1.28	0.90	0.68	6.57*	--	--
2007	0.98	0.04	0.88	9.54**	0.002	27.86**

*$P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$
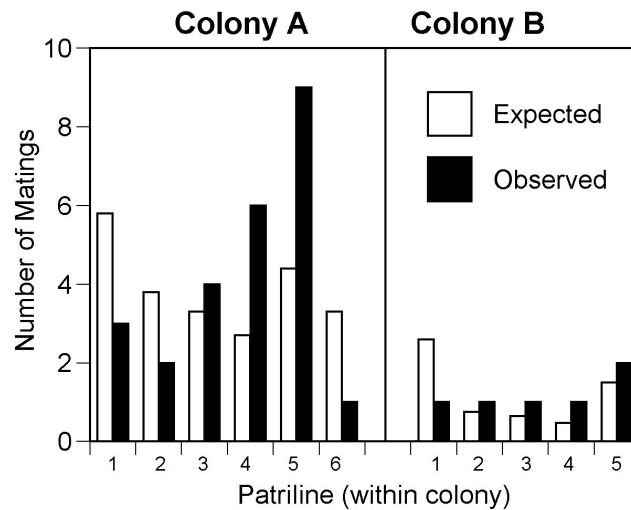
Figure 3.3: Expected and observed numbers of matings differed significantly among patrilines within colonies (chi-square test; $P = 0.001$) for *V. maculifrons* gynes collected in 2007.

Discussion

Female Size Influences Overwintering Survival

We detected a consistent and strong positive relationship between gyne body size and overwintering survival across years (Fig. 3.1, Table 3.1). Our finding that larger gynes survive longer is in line with previous studies examining the effect of female size and survival in several species (STEARNS 1992). For example, queen size was positively associated with queen survival during colony founding in the harvester ant *Pogonomyrmex occidentalis* (WIERNASZ & COLE 2003); heavier *Polistes chinensis* wasp foundresses constructed larger nests (KUDO *et al.* 2005). Additionally, queen size affects dispersal abilities in many social insects, with larger queens generally dispersing farther than smaller queens (KELLER & PASSERA 1989; SUNDSTRÖM 1995; RÜPPELL *et al.* 1998; RÜPPELL & HEINZE 1999; PEETERS & ITO 2001). We note, however, that queen body size is not always correlated with survival, as queen body mass did not affect queen mortality during solitary colony founding in the fire ant *Solenopsis invicta* (BERNASCONI & STRASSMANN 1999). Nevertheless, it seems logical to conclude that queen size frequently directly impacts a queen's ability to survive both overwintering and colony founding, and is therefore strongly associated with female fitness.

Female Size Influences Mating Success

Often traits associated with female survival and fecundity also affect gyne mating success (BONDURIANSKY 2001). In *V. maculifrons,* gyne body length influences mating success (Fig. 3.2, Table 3.2). We found that longer gynes were significantly more likely to be mated in the first and third year of our three-year study. However, shorter gynees were significantly more likely to be mated in the second year. In addition, we discovered that gynes sired by different males varied in the frequency with which they were mated, suggesting an effect of gyne genotype on gyne mating success. Thus our data suggest

that the factors influencing gyne mating success are complex, and that selective pressures may not be consistent across years. Consequently, caution should be used when interpreting data on mating behaviors based on only a single reproductive season or on a limited number of crosses.

Regardless, the significant association between gyne body length and mating success in all three years suggests the importance of gyne length in this mating system. An association between female size and fecundity has been documented in many animal taxa (STEARNS 1992). Among social insects, queen weight was found to be positively associated with queen mate number in the ant *Lasius niger* over two mating seasons (FJERDINGSTAD & KELLER 2004). Therefore, the association between gyne length and both overwintering survival and mating success suggests that gyne length is an honest indicator of female fitness in *V. maculifrons*.

Males prefer to mate with larger, higher fitness females in a wide range of invertebrate and vertebrate species (reviewed by BONDURIANSKY 2001). Male mate choice is predicted to evolve in systems in which 1) males invest valuable and limited resources during mating, such as nuptial food gifts or spermatophores, 2) males can easily locate mates, and 3) females show high variation in quality (BONDURIANSKY 2001). Under these conditions, males may preferentially mate with females displaying traits associated with fecundity such as weight or size (STEARNS 1992). Male mate choice, like other forms of selection, can therefore affect the evolution of physical and behavioral traits in females.

Several features of social insect life histories suggest that males in some species may be selected to practice mate choice. For example, males of most hymenopteran social insects are short-lived (dying after their brief mating period), which results in limited mating opportunities (DUMSER 1980; HÖLLDOBLER & BARTZ 1985). Hymenopteran males also typically undergo testes degeneration early in adulthood, which makes sperm

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a valuable and limited resource (HÖLLDOBLER & BARTZ 1985). Additionally, social hymenopteran males often lek in large groups to which females are attracted, or group around nest entrances and wait for females to exit the nest for mating flights (THORNHILL & ALCOCK 2001). This suggests that mate location is a fairly low-cost activity. We do note that though the operational sex-ratio for many social insects is strongly male-biased, the natural mating behavior and mate-search strategies for *V. maculifrons* are largely unknown, making it difficult to ascertain the cost of mate search in this species. Finally, in many social insects, there is a delay between mating and the production of sexual offspring by the mated queen. During this delay there are many opportunities for queen mortality or colony failure (SPRADBERY 1973; HÖLLDOBLER & WILSON 1990). A male's reproductive fitness is therefore dependent on his mate's ability to survive for a substantial period of time. Thus many features of social insect life history suggest that males could display pre-copulatory male mate choice by preferring to mate with gynes most likely to survive to reproduction (i.e. higher quality females).

We suggest the possibility that gyne size may influence female mating success in social insects, due to males preferentially mating with larger, higher fitness gynes. Other social insect studies are suggestive of male mate choice, though perhaps based on traits other than gyne size. For example, *Iridomyrmex humilis* ant queens mate with multiple males, but most, or possibly all, of the sperm from those matings originate from a single male (KELLER & PASSERA 1992). Therefore, males may be choosing to transfer sperm to particular gynes in that species. In addition, males and gynes of the ant *Leptothorax gredleri* did not necessarily mate with the first individual they met, but rather appeared choosy (OBERSTADT & HEINZE 2003). Moreover, SAUTER & BROWN (2001) found that *Bombus terrestris* males were less likely to mate with gynes that had already mated than with virgin gynes. Consequently, these studies suggest that gyne traits may affect male mating preferences in social insects.

Observations of mating behavior in *Vespula* suggest that males initiate mating attempts (SPRADBERY 1973; ROSS 1983; KOVACS *et al.* 2008) and thus may have the opportunity to make decisions concerning with which gynes they mate. Additionally, males may have an opportunity to judge gyne size as they approach for mating attempts. It is of course crucial to note that gynes also display important behaviors while mating. For example, gynes are capable of dislodging males during mating attempts. Thus we fully acknowledge the possible role and importance of gyne behaviors in mating interactions. Female choice, mutual mate choice, or overall increased female activity do not represent mutually exclusive hypotheses and can all result in the association between gyne size and mating success that we observed in this study. Indeed, successful copulations almost always involve some cooperative behaviors and signals between the two sexes (THORNHILL & ALCOCK 2001).

Nevertheless, we suggest that male mate choice remains a plausible and relatively unexamined explanation. Moreover, social insects present a unique set of life-history traits in which to examine sexual selection, because social insect males are somatically short-lived and, once mated, irreversibly committed to their mates. Most importantly, our study, along with those cited above, suggests that males may play a larger role in social insect mating systems than previously thought and do not necessarily mate with any gyne which they encounter.

Mating Status Does Not Influence Overwintering Survival

Mating can have a negative impact on female survival (EBERHARD 1996; JOHNSTONE & KELLER 2000; SIMMONS 2001). Such negative effects arise because males may attempt to prevent female remating by transferring seminal toxins during ejaculation or otherwise may injure females during mating. Surprisingly, however, we discovered no significant association between gyne matedness and overwintering survival. This lack of

association may arise because males may not be selected to block remating by harming their mates in species such as those in the social Hymenoptera where there is a long delay between mating and reproduction. Under such circumstances, strategies to prevent female promiscuity that would injure the female should be constrained, because males may decrease their own fitness by decreasing the longevity of their mate (BOOMSMA & RATNIEKS 1996; STRASSMANN 2001).

Empirical studies performed in social insects investigating the effects of insemination on queen survival have returned mixed results. In *Bombus terrestris*, virgin gynes survived overwintering longer than naturally mated gynes (GREEFF & SCHMID-HEMPEL 2008). In addition, *B. terrestris* gynes artificially inseminated with sperm from multiple males suffered from higher mortality during overwintering than those inseminated with sperm from a single male, suggesting a negative effect of mating on queen viability (BAER 2005). In contrast, SCHREMPF *et al*. (2005) found that queens mated with either one fertile or one sterilized male enjoyed greater longevity than unmated queen, indicating that mating is actually beneficial to queens in this species.

Genetic Factors Affect Mating Success But Not Overwintering Survival

We uncovered a genetic influence on gyne mating success; gynes belonging to particular patrilines within colonies were mated more often than gynes belonging to other patrilines (Fig. 3.3, Table 3.2). However, patriline did not affect overwintering survival, the other fitness trait measured in this study (Table 3.1). Thus, it is possible that patriline affects female fitness either through a phenotypic trait or life-history event not measured in this study.

Alternatively, this genotypic mating preference may not be universally shared, but rather, specific to particular male-female interactions, similar to genetic incompatibility but assessed prior to copulation. Under the genetic compatibility hypothesis, a female

mates with multiple males in order to reduce the chances of mating with a male whose genotype, in combination with her own, would produce low-fitness offspring (ZEH & ZEH 1997). Related effects have already been noted in the harvester ant, *Pogonomyrmex rugosus*, where different female-male mating combinations led to the production of different proportions of queen and worker castes (SCHWANDER & KELLER 2008).

Our study did not include enough male and female patrilines to determine whether mating success was dependent on joint male and female genotypes. However, such interactions could help explain our unexpected negative association between gyne size and matedness in 2006. That is, important genetic interactions between the sexes may have overridden the predicted positive association between gyne size and mating success.

Regardless, the presence of genetic interactions could have important consequences for gyne mating behaviors. In particular, if the observed patriline effect on gyne mating success was the result of male by female genetic interactions, then it might be advantageous for gynes to increase the number of patrilines represented among their offspring by mating with multiple males under certain conditions (CROZIER & FJERDINGSTAD 2001). Under this scenario, polyandry would allow for a queen to produce more genetically variable daughters. If the environments in which these daughters founded colonies were highly variable, then greater genetic diversity would increase the probability of some daughter queens successfully founding colonies, as well as potentially lowering competition between daughters (CROZIER & PAGE 1985).

Conclusions

Our findings reveal the importance of gyne size in both overwintering survival and mating success in the social wasp *V. maculifrons*. These results suggest that gyne length, particularly gaster length, can act as a cue of female fitness and therefore is

expected to be under natural selection. Additionally, gyne length may also be subject to sexual selection due to male mate choice. Though we realize the need for further studies, particularly those attempting to isolate gyne behaviors, we feel that our findings, especially when combined with those in other social insects (ABELL *et al.* 1999; WIERNASZ *et al.* 2001; BAER 2003; FJERDINGSTAD & KELLER 2004; BAER 2005; FORTELIUS 2005; BAER & BOOMSMA 2006; MERCIER *et al.* 2007; OPPELT & HEINZE 2007; VISICCHIO *et al.* 2007), provide evidence for the role of natural selection as well as pre-copulatory sexual selection in the evolution of morphologies and behaviors in social insects.

Acknowledgements

CHAPTER 4[3]

ENVIRONMENTAL AND GENETIC INFLUENCES ON QUEEN AND WORKER BODY SIZE IN THE SOCIAL WASP *VESPULA MACULIFRONS*

Abstract

Many social insects exhibit morphologically distinct worker and queen castes that perform different functions. These functional differences generate unique selection regimes, which may differentially affect levels of genetic variation associated with phenotypic variation in the two castes. This study sought to determine if the genetic variation underlying phenotypic differences varied between the worker and queen castes of the social wasp *Vespula maculifrons*. We determined the effects of genotype on worker and queen body size by 1) calculating broad and narrow sense heritabilities for several morphological traits, 2) examining whether some paternal genotypes produced more morphologically diverse offspring than others, and 3) determining whether trait size variation was associated with genetic variation within colonies. Few morphological traits were significantly heritable, indicating that little genetic variance for those traits existed within our study population. Moreover, most estimates were not consistently significant across multiple study years. We also found that some patrilines produced more morphologically variable offspring than others, suggesting a role of genotype in phenotypic plasticity. And finally, no significant correlations between genetic diversity arising from multiple mating by queens within colonies and trait variation in either caste were found. Overall, our findings indicate a weak effect of genotype on both worker and

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gyne body size variation and are suggestive of a large environmental influence on morphological trait size. Moreover, our results do not indicate that levels of genetic variation underlying size variation differ substantially between castes in this species.

Introduction

Social insects often display body size variation between their female castes. Specifically, the two major female castes in hymenopteran social insects consist of fully fertile queens and subfertile workers (HÖLLDOBLER & WILSON 1990). In most taxa, queens are considerably larger than workers. These size differences likely reflect the different selective pressures encountered by the two castes which arise because of their distinct societal functions and life-histories. For instance, worker size often determines function within a colony (OSTER & WILSON 1978; WHEELER 1986b; 1991; HÖLLDOBLER & WILSON 1990; FJERDINGSTAD & CROZIER 2006). Larger workers frequently undertake tasks such as colony defense or food processing, whereas smaller workers may be more efficient at completing tasks like brood tending. Thus producing a diversity of worker body sizes may be selectively advantageous for a queen and her colony. In contrast, queen body size may be under directional selection. Large body size may increase a queen's ability to survive colony founding as well as maintain a colony through to the production of new sexuals, particularly in species where queens found colonies independently (ABELL et al. 1999; PEETERS & ITO 2001; DEHEER 2002; WIERNASZ & COLE 2003; BARGUM et al. 2004; FJERDINGSTAD 2005).

If the opposing selective pressures operating in the two castes are strong enough, then differences in the genetic architecture controlling body size may result (LANDE 1980; ARCHER et al. 2003; PHELAN et al. 2003; BONDURIANSKY & ROWE 2005). Specifically, the different selective pressures operating on body size in the two castes may lead to differences in the magnitude of genetic variation underlying body size

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variation. For example, an absence of directional selection on worker body size would result in the maintenance of more genetic variation for worker body size. In contrast, little genetic variation is expected to underlie phenotypic differences in queen size, because such variation is expected to have been erased by the operation of directional selection over time. Therefore, since workers will be less likely to be under the same type of directional selection as queens, we predicted that there would be greater genetic control of worker body size than queen body size.

To test this prediction, we used classical quantitative genetic methods to estimate broad and narrow sense heritabilities for several worker and queen traits in a social wasp. Heritability estimates provide information on the amount of genetic variation underlying phenotypic variation and can be used as indicators of the selective pressures that have acted on traits in the past (ROFF 1997). Generally, strong, multi-generational directional selection will erode additive genetic variance from a population, which will result in low narrow sense heritability (FALCONER & MACKAY 1996). Therefore, we predicted that queens would display lower heritability for size than workers.

In addition to investigating the relative heritability of traits between castes, we were also interested in determining if genotype affected *variation* in trait size. We tested whether some genotypes produced more variable offspring than others, which would suggest that some genotypes were more phenotypically plastic than others. Again, due to the presumed directional selection faced by queens, we predicted that these phenotypically plastic genotypes would be expressed more often among workers than queens.

Finally, we examined whether increased genetic diversity within a colony resulted in increased phenotypic variation among the worker and queen castes. Genetic diversity may lead to phenotypic diversity if genotype influences morphology (OSTER & WILSON 1978). We predicted a positive correlation between genetic diversity and morphological

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variation within colonies for workers because we expected genetic variation to influence phenotypic variation in the worker caste. However, we did not predict such a correlation for queens because we did not expect genetic variation to strongly influence phenotypic variation in the queen caste (CROZIER & FJERDINGSTAD 2001).

We chose to investigate the influence of genotype on body size in the social wasp *Vespula maculifrons. Vespula maculifrons* is a convenient taxon for investigating genetic effects on size for several reasons. First, workers and queens show strongly divergent morphology and behavior and therefore may be expected to be under distinct types of selection as discussed above (*V. maculifrons* natural history reviewed by SPRADBERY 1973; MACDONALD & MATTHEWS 1981; GREENE 1991). Second, *V. maculifrons* colonies produce abundant workers and new queens each year, thus providing the material necessary for genetic studies. Additionally, the social structure displayed by *V. maculifrons* is well understood; colonies are headed by a single multiply mated queen (range in queen mate number of 3-9; ROSS 1985; GOODISMAN *et al.* 2007a), and members of all patrilines are found in both castes (GOODISMAN *et al*. 2007b). The variation in mate number found across colonies provides the genetic variation necessary to undertake our study examining how size varies among genotypes.

Methods

Sample Collection

Between the months of July and November in 2004, 2005, and 2008, a total of 61 *V. maculifrons* colonies were collected near metropolitan Atlanta, Georgia, United States (2004 *N* = 33; 2005 *N* = 4; 2008 *N* = 24). All colonies contained workers and sixteen colonies also contained newly eclosed queens that had not yet mated (hereafter referred

to as gynes; 2004 N = 7; 2005 N = 4; 2008 N = 5). In 2004, twelve collected colonies also contained the true reproductive foundress of the colony (hereafter referred to as the queen, cf. with gyne above). In 2008, the queen was identified in five colonies containing adult gynes, and an additional 19 queens were identified in colonies containing only adult workers. Thus, in total, 1585 workers ($\bar{x} \pm$ SD; 44.03 $\pm$ 25.23 workers per colony), 434 gynes (39.45 $\pm$ 1.51 gynes per colony), and 36 queens were collected and used for analyses in this study.

Genetic analysis

DNA was extracted from single legs of workers and gynes. Tubes containing the leg samples were submerged into liquid nitrogen for no more than 30 seconds. The legs were then immediately crushed using plastic pestles. Five hundred µl of 5% Chelex solution was added to the samples, which were then incubated at 95º C in a heat block for 20 minutes (WALSH *et al.* 1991).

The worker genotypes were determined at eight microsatellite loci: LIST2003, LIST2004, LIST2013, LIST2019, LIST2020 (DALY *et al.* 2002), RUFA 5 (FOSTER *et al.* 2001), VMA-3, and VMA-6 (HASEGAWA & TAKAHASHI 2002). The heterozygosities at these loci were sufficiently high so that the probability of two males having the same genotype (nondetection error; BOOMSMA 1996) was low (<< 0.0001; GOODISMAN *et al.* 2007b). Gyne genotypes were determined at a subset of loci (LIST2004, LIST2013, LIST2019, LIST2020, and VMA-6) that possessed sufficient variation to distinguish all patrilines within colonies.

All PCRs were conducted in a volume of 15 µl containing a final concentration of 4mM MgCl$_2$, 0.2 mM dNTPs, 1X PCR buffer, and 0.1 U Taq DNA polymerase. In addition, six of the eight loci were multiplexed in pairs. Pair 1 consisted of the loci LIST2020 and VMA-6 combined at final primer concentrations of 0.47 µM for each locus.

Pair 2 included RUFA 5 and VMA-3 combined at final primer concentrations of 0.8 µM

and 0.13 µM, respectively. Pair 3 consisted of LIST2019 and LIST2013 combined at final

primer concentrations of 0.33 µM and 0.67 µM, respectively. Finally, both LIST2004 and

LIST2003 were amplified singly at final primer concentrations of 0.67 µM. The PCR cycle

was run as follows: 2 min denaturation at 94º C, 30 cycles of 94º C for 30 seconds,

primer optimized annealing temperatures (pair 1 = 60° C; pair 2 = 54° C; pair 3 = 60° C;

LIST2004 = 60° C, LIST2003 = 48° C) for 30 seconds, 72º C for 30 seconds, and a final

72º C extension for 10 minutes. PCR samples were run on 2.5% agarose gels to confirm

amplification. PCR products were visualized using an Applied Biosystems PRISM® 3100

Genetic Analyzer. Gynes and workers were assigned to patrilines within colonies based

on their multilocus genotype as described by HOFFMAN *et al.* (2008).

Morphological measurements

After DNA extractions were completed, workers from 37 colonies (2004 N = 33;

2005 N = 4) and gynes from eleven of those colonies (2004 N = 7; 2005 N = 4) were

dried at 60° C. Individual dry mass was then recorded (workers from 10 of the colonies

collected in 2004 were not photographed and were therefore analyzed for dry mass

only). Additionally, digital photos were taken of workers from 46 of the collected colonies

(2004 N = 23; 2005 N = 4; 2008 N = 19), gynes from a total of 16 colonies (2004 N = 7;

2005 N = 4; 2008 N = 5), and the queens from 36 colonies (2004 N =12; 2008 N = 24).

Each individual was photographed in a dorsally facing position with a Zeiss©

millimeter micrometer to allow for calibration. We measured the linear dimensions of

each of the following six traits: thorax width measured from the inside of the left wing

tegula to the inside of the right wing tegula, i.e. the length of the mesoscutum (TW),

thorax length (TL), length from the head to the end of the thorax (HTL), length of the

third tergum (3TL), gaster length (GL), and total body length (BL). The length of each of these traits was measured using tools within the Canvas 9.0.4 computer package.

For this study we were particularly interested in the effects of genotype on general body size (represented by Mass and BL). Significant correlations have been found between both of these traits and survival and fecundity in a variety of social insects (RÜPPELL *et al.* 1998; ABELL *et al.* 1999; PEETERS & ITO 2001; DEHEER 2002; WIERNASZ & COLE 2003; BARGUM *et al.* 2004; FJERDINGSTAD 2005). The other measured traits represent components of overall body size. Moreover, several of these traits are likely to be of functional importance in one or both castes. For example, a recent studies suggest that length (i.e., GL and BL) of *V. maculifrons* gynes is correlated with mating success (KOVACS *et al.* 2008; unpublished). In addition, thoracic measurements (TW and TL) may be related to flight capabilities (MARDEN 2000).

Statistical Analyses

Broad sense heritabilities

Broad sense heritability (H^2) is defined as the proportion of the total variation that can be attributed to genetic variation within a population, or the ratio of genetic variance (V_G) to total phenotypic variance (V_P; FALCONER & MACKAY 1996). H^2 was estimated using a sire-within-dam setup modified for haplodiploid organisms for both worker and gyne traits (Table 2 in FJERDINGSTAD 2005). Information resulting from two-factor nested ANOVAs (patriline within colony, and colony) was used to estimate H^2 for measured traits in each year separately due to significant differences between years (see below).

We effectively assumed that epistatic variance makes a negligible contribution to our estimates of broad sense heritability due to difficulties in calculating epistatic variance in breeding systems (FALCONER & MACKAY 1996). Therefore, only the combined

additive and dominance components were calculated. Estimates of broad sense

heritability for all gyne and worker traits were calculated as twice the patriline variance

($\sigma^2_{patriline} = \frac{1}{2}V_A + \frac{1}{2}V_D$) divided by the total variance ($\sigma^2_{total} = V_A + V_D + V_{EC} + V_{EW}$;

FJERDINGSTAD 2005). H^2 estimates were deemed to be significant when the lower 95%

confidence interval did not include zero (FALCONER 1989).

In addition to determining the broad sense heritability for gyne and worker

morphological traits, we estimated the proportion of the observed variance explained by

each level (i.e. colony, patriline within colony, and offspring within patriline) by dividing

the observational pure component of variance (σ^2) by the total variance (σ^2_{total}) and

multiplying by 100 (SOKAL & ROHLF 1995). We also used a three-factor nested ANOVA

to determine the influence of patriline within colony within sampling year, colony within

sampling year, and sampling year on trait size.

Narrow sense heritabilities

Narrow sense heritability (h^2) is an expression of the extent to which offspring

phenotypes are determined by genes transmitted from the parents, and is expressed by

the ratio V_A / V_P. h^2 can be estimated as twice the slope (*b*) of the regression of mean

offspring size on the mothers' sizes (parent-offspring regression). Narrow sense

heritabilities could be calculated for morphological traits for both workers and gynes

belonging to colonies that were collected with their mother queen. However, when

offspring and parent differ in trait size, as is the case for traits in workers and queens,

then trait sizes must be standardized (LYNCH & WALSH 1998). Therefore, prior to

analyses, both worker and gyne offspring as well as queen trait sizes were standardized

to a mean of zero and unit variance within each year. These standardized trait sizes

were then used to estimate h^2.

Influence of genotype on variation in trait size

In addition to determining if workers and gynes produced by different males differed in mean trait size, we also investigated whether the *variance* for each trait differed among workers and gynes sired by different males. To explore this issue, we tested if the variances in trait size for each patriline were equal within each colony separately using Levene's test for equality of variances. We then combined the information by performing a *Z*-transform test on the colony *P*-values to determine overall significance across all colonies (WHITLOCK 2005).

Influence of genetic variability on morphological variability within colonies

To test for an effect of queen mate number on phenotypic variation among both worker and gyne offspring, we used Spearman's correlation coefficients to determine if the standard deviation for each trait was directly related to the effective number of times the queen of each colony mated (k_{e3}) in 2004. Effective paternity (k_{e3}) for each colony was calculated using the sample size correction proposed by NIELSEN *et al.* (2003). k_{e3} combines information on the number of times a queen mates and the unequal contributions of her mates to their offspring, thereby providing a measure of the amount of genetic variability of diploid individuals within colonies (BOOMSMA & RATNIEKS 1996).

Results

A total of 1824 individuals were assigned to patrilines within 36 colonies; eleven of these colonies contained both workers and gynes (workers *n* = 1427; gynes *n* = 397).

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Table 4.1: Differences in the sizes of *V. maculifrons* worker and gyne traits among years, among colonies within year, and among patrilines within colony. Significant *P*-values are in bold. Gyne traits differed significantly between patrilines more than worker traits.

Trait	Factor	Workers			Gynes		
		F	*df*	*P*	*F*	*df*	*P*
Thorax Width	Year	0.89	1	0.346	3.27	1	0.071
	Colony (Year)	8.17	15	**0.0001**	5.34	9	**0.0001**
	Patriline (Year, Colony)	0.92	79	0.665	1.86	59	**0.0004**
Thorax Length	Year	0.367	1	0.545	6.24	1	**0.013**
	Colony (Year)	8.94	15	**0.0001**	2.55	9	**0.008**
	Patriline (Year, Colony)	1.05	79	0.370	0.96	59	0.567
Head to Thorax Length	Year	0.01	1	0.940	0.88	1	0.348
	Colony (Year)	4.69	15	**0.0001**	2.95	9	**0.002**
	Patriline (Year, Colony)	1.00	79	0.488	1.42	59	**0.030**
Third Tergum Length	Year	0.76	1	0.384	2.59	1	0.109
	Colony (Year)	7.92	15	**0.0001**	1.63	9	0.105
	Patriline (Year, Colony)	1.22	79	0.108	1.13	59	0.257
Gaster Length	Year	6.00	1	**0.015**	85.07	1	**0.0001**
	Colony (Year)	13.46	15	**0.0001**	7.08	9	**0.0001**
	Patriline (Year, Colony)	1.15	79	0.182	1.45	58	**0.026**
Body Length	Year	1.58	1	0.209	65.73	1	**0.0001**
	Colony (Year)	8.94	15	**0.0001**	6.43	9	**0.0001**
	Patriline (Year, Colony)	0.87	79	0.771	1.11	58	0.289
Mass	Year	110.53	1	**0.0001**	111.58	1	**0.0001**
	Colony (Year)	20.76	34	**0.0001**	6.11	9	**0.0001**
	Patriline (Year, Colony)	1.31	167	**0.008**	1.38	59	**0.045**

The means of all seven worker traits differed significantly between colonies ($P < 0.0001$

for all worker traits; Table 4.1), while only two worker traits (GL and Mass) differed

significantly between years (Table 4.1). Most gyne traits also differed significantly

between colonies ($P < 0.05$ for all gyne traits except 3TL; Table 4.1), as well as years (P

< 0.05 for gyne traits except TW, HTL, and 3TL; Table 4.1). These results suggest that

environmental variation associated with the development of different colonies, genetic

variation associated with colony makeup, or interactions of these factors generated size

variability between colonies.

Broad sense heritabilities

        To gain a better understanding of whether genetic variation specifically affected

phenotypic variation, we quantified broad sense heritabilities (H^2) for all traits. Mass in

2004 was the only trait with an H^2 estimate significantly greater than zero in workers

(Table 4.2). In contrast, H^2 estimates for TW, GL, and Mass in 2004 and TW and BL in

2005 were significantly different from zero in gynes (Table 4.2). The lower 95%

confidence intervals for all other worker and gyne H^2 estimates contained zero and

therefore were not considered significant (Table 4.2). Thus genetic variation contributed

through male sires had only weak effects on size variation in the two castes.

        The proportion of variance explained by colony was greater than that explained

by patriline within colony for all worker traits, and most gyne traits (Table 4.2). Most

notably the percentages of variance explained by offspring within patrilines were much

larger than both the percentages of variance explained by either colony or patriline within

colony for both the worker and gyne castes (Table 4.2). This suggests a high proportion

of the observed variance in trait sizes in both castes is due to non-genetic factors.

Table 4.2: Proportion of variance in worker and gyne trait size explained at different levels (colony, patriline, and within patriline among offspring), and estimates of broad sense heritability ($H^2 \pm$ SE) in *V. maculifrons*. Significant H^2 estimates are in bold. Few worker or gyne traits displayed significant H^2 estimates, suggesting a limited role of genotype in determining body size in both female castes.

| Trait | Level | Workers | | | | Gynes | | | |
| | | % variance explained | | H^2 | | % variance explained | | H^2 | |
		2004	2005	2004	2005	2004	2005	2004	2005
Thorax Width	Colonies	12.21	27.24	0.002 ± 0.03	-0.09 ± 0.06	4.71	8.51	**0.20 ± 0.07**	**0.46 ±**
	Patriline	0.10	-4.73			9.99	22.81		**0.14**
	Offspring	87.69	77.49			85.30	68.68		
Thorax Length	Colonies	14.45	22.86	0.04 ± 0.03	-0.12 ± 0.06	1.02	8.60	0.05 ± 0.06	0.04 ±
	Patriline	1.83	-6.12			2.52	2.00		0.08
	Offspring	83.72	83.25			96.45	89.40		
Head to Thorax Length	Colonies	8.23	6.43	0.03 ± 0.03	-0.22 ± 0.06	6.80	5.71	0.02 ± 0.05	0.06 ±
	Patriline	1.73	-11.21			0.80	2.81		0.09
	Offspring	90.04	104.79			92.40	91.48		
Third Tergum Length	Colonies	9.32	36.50	0.07 ± 0.04	-0.03 ± 0.06	2.69	-1.19	-0.01 ± 0.05	0.12 ±
	Patriline	3.52	-1.74			-0.68	6.06		0.10
	Offspring	87.16	65.24			98.00	95.13		
Gaster Length	Colonies	21.95	25.04	0.02 ± 0.03	0.06 ± 0.08	13.55	-0.49	**0.13 ± 0.06**	0.20 ±
	Patriline	1.01	3.12			6.37	9.98		0.11
	Offspring	77.04	71.84			80.07	90.51		
Body Length	Colonies	16.33	18.66	0.001 ± 0.03	-0.11 ± 0.07	12.71	-1.52	-0.04 ± 0.04	**0.38 ±**
	Patriline	0.04	-5.73			-1.76	18.91		**0.14**
	Offspring	83.62	87.07			89.04	82.60		
Mass	Colonies	31.88	29.70	**0.06 ± 0.02**	0.02 ± 0.07	9.59	4.42	**0.18 ± 0.06**	-0.07 ±
	Patriline	2.83	1.13			8.90	-3.43		0.07
	Offspring	65.29	69.17			81.51	99.01		
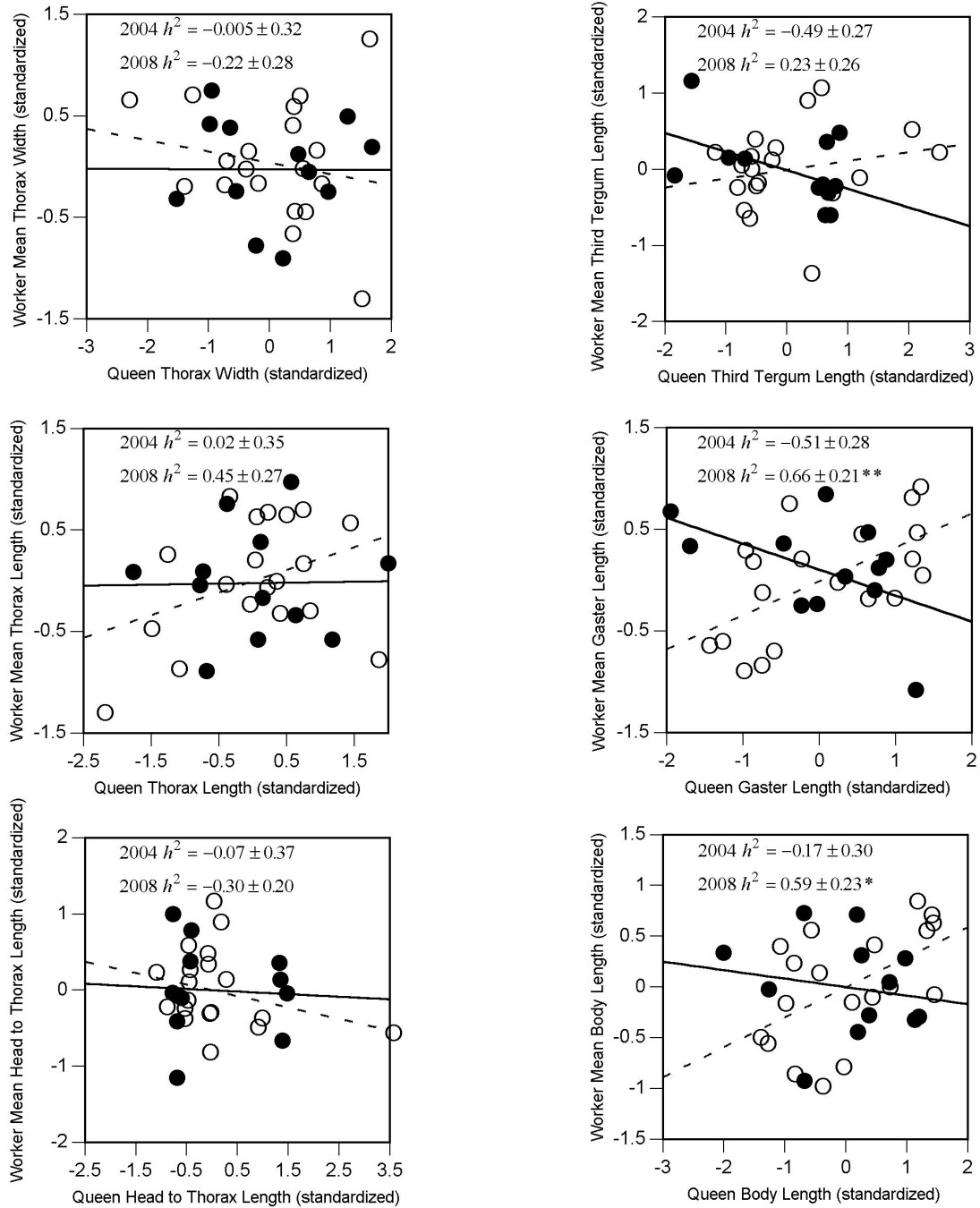
Figure 4.1: Narrow sense heritabilities ($h^2 \pm$ S.E.) for worker morphological traits in 2004 (filled circles, solid lines) and 2008 (open circles, dotted lines). 2005 worker gaster length and body length h^2 estimates differed significantly from zero, as indicated by asterisks (* $P < 0.05$, ** $P < 0.01$).

Narrow sense heritabilities

Offspring phenotypes can be influenced by both dam and sire genotypes. We quantified the effect of dam genotype on offspring phenotype by calculating narrow sense heritabilities for worker and gyne traits for colonies collected with the queen. We found that maternal queen phenotype had little correlation with mean worker phenotype, resulting in mostly non-significant h^2 estimates in both 2004 and 2008 (Figure 4.1; in 2008 only worker GL and BL displayed significant heritabilities). Interestingly, worker h^2 estimates showed large differences between years, in both direction (negative or positive) and significance. In fact, the parent-offspring regressions for GL were significantly different between years (ANCOVA, $F_{3,27}$ = 4.47, P = 0.01). The association between queen size and gyne size was also investigated for five colonies collected in 2008. No gyne traits displayed significant h^2 estimates (Figure 4.2), although our sample size was very limited for these calculations. Thus, overall, the genetic contribution to both gynes and workers through their mother seemed to be weak.

Paternal affect on variation in trait size

Genotype, rather than just influencing mean trait size, may also affect the amount of variability in trait size. That is, individuals sired by different males may differ in the variability of their traits, with individuals from some patrilines displaying a wider range of trait sizes than those from others (i.e. greater phenotypic plasticity). Our analyses revealed significant differences in the amount of variation displayed by both workers and gynes sired by different males for several traits (Table 4.3). Worker Mass in 2004 as well as worker 3TL in both years showed significantly different amounts of variation between patrilines (Table 4.3). Among gyne traits, Mass in both 2004 and 2005 and TW and HTL in 2005 displayed significantly different levels of variation between patrilines. Thus, certain genotypes do lead to more variable phenotypes than others.
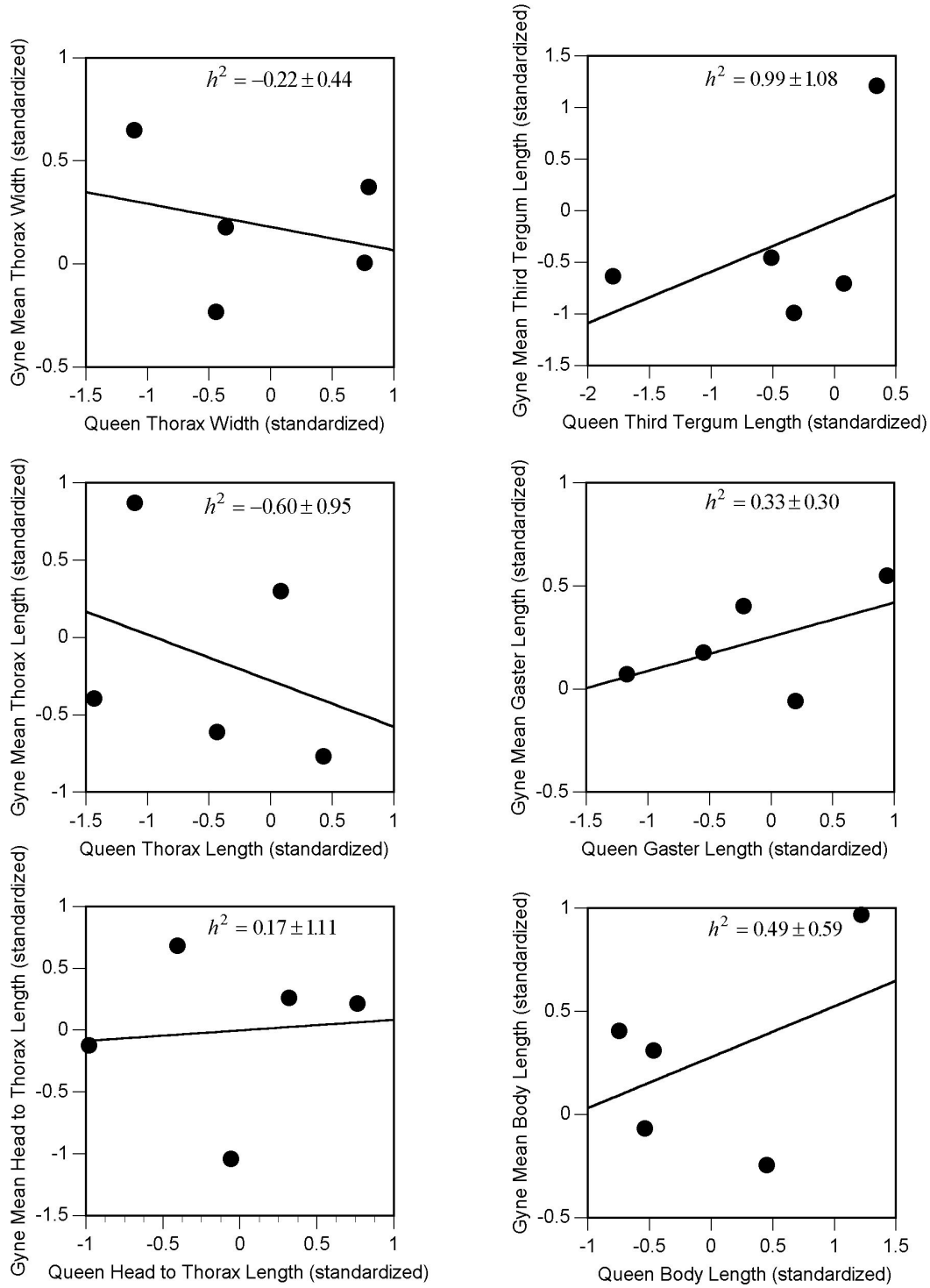
Figure 4.2: Narrow sense heritabilities ($h^2 \pm$ S.E.) for gyne morphological traits in 2008. No gyne traits showed significant narrow sense heritability.

Table 4.3: Significance levels (*P*) for tests investigating if *V. maculifrons* workers and gynes sired by different males differed in the amount of variation displayed. Significant *P* values are in bold. Some worker and gyne traits displayed significant amounts of variation between patrilines, suggesting that certain genotypes may result in more variable phenotypes than others.

	Significance of *(P)* of differences in variances among patrilines			
	Workers		Gynes	
Trait	2004	2005	2004	2005
Thorax Width	0.351	0.124	0.364	**0.016**
Thorax Length	0.201	0.563	0.504	0.690
Head to Thorax Length	0.542	0.547	0.708	**0.032**
Third Tergum Length	**0.002**	**0.002**	0.318	0.137
Gaster Length	0.106	0.145	0.074	0.226
Body Length	0.126	0.101	0.071	0.626
Mass	**0.0001**	0.151	**0.008**	**0.012**

Effect of genetic variability on trait variation

A queen increases the genetic variation among her offspring by mating multiply. A possible consequence of multiple mating is increased size variation among offspring if size has a genetic basis. However, no worker or gyne trait standard deviations were significantly correlated with queen effective mate number (Figure 4.3, Spearman's correlations, $P > 0.05$ for all traits). Thus, total genetic variation found among colony mates does not appear to affect total phenotypic variation in *V. maculifrons* colonies.

Discussion

Social insect colonies are often composed of individuals belonging to distinct worker and queen castes. To determine the effects of genotype on variation in worker and queen body size and to test predictions regarding the nature of genetic variation underlying morphological traits, we 1) calculated broad and narrow sense heritabilities for several morphological traits in both castes of a social wasp, 2) examined whether some paternal genotypes produced more morphologically diverse offspring than others, and 3) determined whether there was a positive correlation between trait size variation within castes and the amount of genetic variation within colonies.

Genetic effects on mean trait size

We found evidence that variation in genotype influenced variation in phenotype for a few worker and gyne traits (Tables 4.1 and 4.2, Fig 4.1; workers: gaster length, body length, and mass; gynes: thorax width, head to thorax length, gaster length, body length, and mass). Interestingly, some of these traits may be associated with individual caste function. For example, thorax width is set early in pupal development and may be linked to flight, which may have important implications for mating and colony founding in

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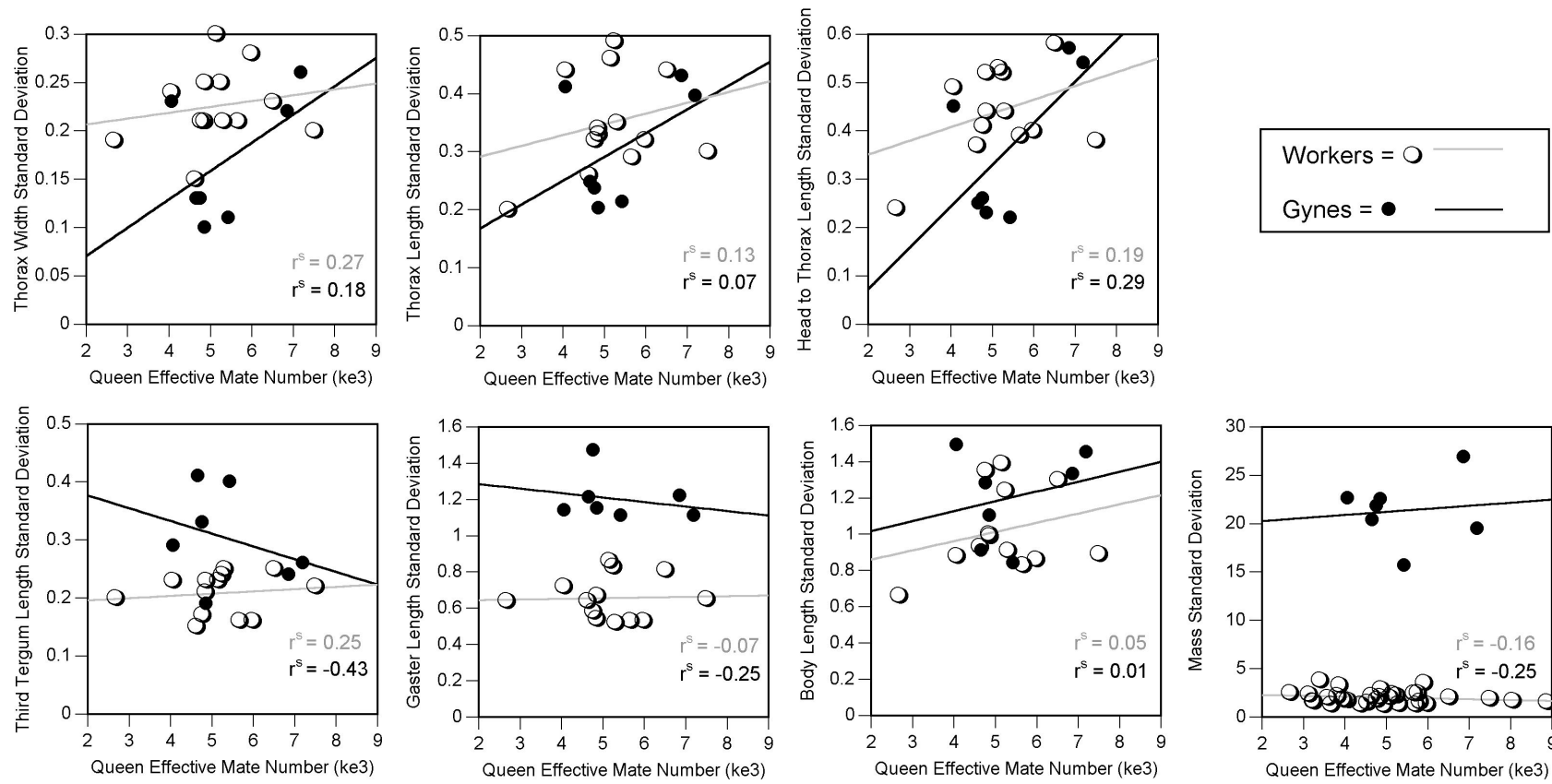
Figure 4.3: Correlations between the amount of genetic variability, as judged by queen effective mate number (k_{e3}) and morphological variability (trait standard deviation) in worker and gyne traits in 2004. No correlations were significantly different from zero.

V. maculifrons. Moreover, a previous study in this species revealed that gyne gaster length and body length were correlated with female mating success (KOVACS *et al*. 2008).

The existence of genetic variation for traits potentially linked to fitness components (i.e. thorax width, gaster length, and body length, especially in gynes) is somewhat surprising, because directional selection may be expected to remove additive genetic variation for that trait from the population (MOUSSEAU & ROFF 1987; FALCONER 1989). However, recent research suggests that the magnitude of heritability for fitness-related traits may not always be so clear due to the influence of non-additive effects and epistatic interactions (WOLF *et al.* 1998; MERILA & SHELDON 1999). Thus, the significant heritabilities for these morphological traits, which may be linked to variation in fitness, may result from dominance and epistatic variance. This hypothesis is bolstered by the fact that all significant heritability estimates for gynes were broad sense estimates, which include dominance variance. If directional selection quickly erodes additive genetic variation for a fitness trait then most of the remaining genetic variation would be due to dominance, and therefore the contribution of dominance is expected to be greater in fitness traits than non-fitness traits (ROFF *et al.* 1997; MERILA & SHELDON 1999). Additionally, some studies have revealed that fitness-related traits are often controlled by more loci than non-fitness related traits (FALCONER 1989; ARMBRUSTER *et al.* 1997) and therefore epistatic interactions may substantially contribute to variation in fitness-related traits (LYNCH & WALSH 1998).

Though heritabilities were significant for a few worker and gyne traits, most traits were not strongly influenced by sire and dam effects. In fact, we uncovered significant and strong differences in mean trait size between colonies for both workers and gynes (Table 4.1 and 4.2). Additionally, more trait variance was explained by differences between colonies, which represent a combination of both maternal and environmental

effects, than by the amount of variance within patrilines (Table 4.2). Thus, variation in individual size in both gynes and workers is largely determined by environmental conditions in *V. maculifrons*.

The heritability estimates calculated for the *V. maculifrons* in this study were generally smaller than those calculated in previous studies of social insects (COLLINS *et al.* 1984; BIENEFELD & PIRCHNER 1990; 1991; POKLUKAR & KEZIC 1994; FRASER *et al.* 2000; RÜPPELL *et al.* 2001; HUGHES *et al.* 2003; BARGUM *et al.* 2004; FJERDINGSTAD 2005; RHEINDT *et al.* 2005; SCHWANDER *et al.* 2005; JAFFE *et al.* 2007; FOURNIER *et al.* 2008). For example, the magnitudes of head width heritabilities for both ant workers and queens range from 0.10 (workers, FOURNIER *et al.* 2008) to 0.88 (queens; RÜPPELL *et al.* 2001). These differ markedly from estimates in *V. maculifrons*, which were generally quite low (Table 4.2; broad sense heritability estimates for gyne thorax width in both years and body length in 2005 being the notable exceptions). One explanation for this result is that the traits measured in this study are more closely related to fitness in wasps than head width, for example, is in ants. Thus, selection may have removed genetic variation from the system, as discussed above. Indeed, estimates of broad sense heritability for body weight, a trait also measured in our current study, in *Lasius niger* queens were non-significant and close to the broad sense heritability estimates obtained in this study (FJERDINGSTAD 2005). This may indicate that similar traits experience similar selective pressures across species.

Another reason for our overall low heritabilities may be indirect genetic effects (WOLF *et al.* 1998). Social insects are defined by the extensive interactions among colony members (HÖLLDOBLER & WILSON 1990). These interactions may result in indirect genetic effects on phenotypic trait expression (WOLF *et al.* 1998) which result when the genes expressed in a social partner affect an individual's phenotype (WOLF *et al.* 1998; WOLF 2000). The interaction of social conspecifics can create a genotype-by-genotype

interaction that in addition to contributing to environmental variance, may also affect non-additive genetic variance (VIA & LANDE 1985; 1987). Under certain conditions these interactions can inflate, mask, or even generate negative heritability estimates (MEFFERT 1995). Due to the design of our study, we were unable to estimate indirect genetic effects, but LINKSVAYER (2006) detected significant maternal and sib-social heritability estimates in ants (see also BIENEFELD & PIRCHNER 1991; RÜPPELL *et al.* 2001; LINKSVAYER & WADE 2005). Thus, social environment likely plays a large role in determining phenotype in social insects and may have a genetic basis as well.

We found a considerable amount of variation in heritability estimates between years, as was the case in previous studies documenting variation in heritability across years or environments (LARSSON *et al.* 1997; MERILA 1997; SGRO & HOFFMANN 1998; BARGUM *et al.* 2004; but see FJERDINGSTAD 2005). This variation between years may be due to changes in either the amount of genetic variance or the total phenotypic variance present in different years and is relatively unsurprising due to the use of a natural population in this study (PRICE & SCHLUTER 1991). Regardless, the observed variation between years suggests that caution be used when interpreting heritability estimates obtained from only a single experiment or breeding season.

Our original motivation for this study was to determine if the influence of genetic variation on phenotypic variation differed between castes. In particular, we expected that variation in worker morphological traits would show evidence of genetic control, and would therefore display considerable amounts of genetic variation and significant heritabilities. However, our results ran somewhat contrary to this prediction. We failed to document substantial amounts of additive genetic variation or strong heritabilities for most worker traits. We also predicted that variation in gyne traits would not be strongly influenced by genotype due to putative positive directional selection on reproductive female size. We did indeed find a general lack of additive genetic control for many gyne

traits, although variation in some traits was significantly influenced by nonadditive

genetic variation. Moreover, more gyne than worker traits were influenced by genetic

variation. Thus our results did not strongly follow our predictions, thereby casting some

doubt on the presumed types of selection acting on morphological traits in social insect

castes.

Genetic affect on variation in trait size

      Vespula maculifrons wasps of all genotypes show considerable levels of

phenotypic plasticity because they can develop into either gynes or workers (GOODISMAN

et al. 2007b). Nevertheless, our analyses revealed significant differences in the *variance*

of trait sizes exhibited by workers and gynes sired by different males (Table 4.3). We

believe that these are among the first analyses to demonstrate a genetic component to

morphological trait variability within social insect castes. However, previous studies in

other nonsocial animal taxa have also found significant heritabilities for plasticity in traits

such as egg laying date and seasonal body mass (NEWMAN 1994; NUSSEY *et al.* 2005a;

2005b; STINCHCOMBE *et al.* 2004; PELLETIER *et al.* 2007; JENSEN *et al.* 2008).

      Our results suggest that some genotypes may be more plastic than others. One

might expect that genotypes showing more phenotypic plasticity would be selectively

advantageous under some circumstances (SCHEINER & GOODNIGHT 1984; SCHEINER

2002). Indeed due to the general life histories of social insects, heritable phenotypic

plasticity for morphological traits may be advantageous since it would allow for colonies

to respond to varying environmental conditions by producing individuals of different

sizes.

Relationship between genetic and phenotypic variation

Both polyandry (female multiple mating) and polygyny (multiple reproductive females within a colony) generally result in increased genetic variation within colonies relative to colonies headed by one, singly mated queen. If genotype has a strong influence on morphology, increased genetic variation may lead to an increase in phenotypic variation. For example, if an increase in phenotypic variation results in increased worker task efficiency, polyandry and polygyny may be selectively advantageous (CROZIER & PAGE 1985; CROZIER & FJERDINGSTAD 2001; FJERDINGSTAD & CROZIER 2006). In fact, FJERDINGSTAD & CROZIER (2006) found a significant negative correlation between relatedness, which is relatively low in colonies headed by multiple reproductives, and variation in worker body mass across 35 ant species supporting the hypothesis that genetic variability is associated with phenotypic variability in social insects.

However, we found no correlation between queen mate number and worker or gyne size variation in *V. maculifrons*. This lack of correlation is not surprising given the generally low heritability estimates of the analyzed traits and the low variation in queen mate number in this species. A lack of correlation between genetic diversity and trait variability has also been reported in a few polyandrous and polygyne ants (BROWN & SCHMID-HEMPEL 2003; SCHWANDER *et al.* 2005). We note that other mechanisms, such as polygyny syndrome resulting from competition among reproductives (KELLER 1993), may lead to the lack of correlation between genetic variation and phenotypic variation within polygyne taxa. However, such explanations cannot explain the lack of correlation between genetic and phenotypic variation in polyandrous taxa. Thus, these data suggest that polyandry would not necessarily evolve in response to selection for a morphologically variable worker force given the present-day association between genotype and worker size in *V. maculifrons*.

Conclusions

This study sought to understand the evolutionary and ecological factors affecting size in social animals by investigating genetic influences on size variation in a natural social wasp population. We determined the genetic effects underlying size variation in *V. maculifrons* workers and gynes, and found a weak effect of genotype, but a strong effect of environment, on variation in most traits. Additionally, we detected significant differences between offspring belonging to different patrilines in the levels of morphological variation expressed, suggesting that some genotypes may be more phenotypically plastic than others. Finally, we uncovered no evidence of correlations between the amount of variation in worker and gyne traits and queen mate number, indicating that high genetic variation within colonies did not necessarily translate into high phenotypic variation among colony mates. Overall, our study suggests a strong environmental influence on morphological trait size in this taxon. We suggest that future research aimed at understanding the effect of genotype on morphology in social insects should investigate a broad range of traits, including those associated with caste and sex function, in order to provide a better picture of which traits are under selection in different phenotypic forms. Overall, such data would help provide insight into the factors that have led to the domination of terrestrial communities by social animals.

CHAPTER 5[4]

ALLOMETRY AND THE GENETIC ARCHITECTURE OF CASTE DIMORPHIC TRAITS IN THE SOCIAL WASP *VESPULA MACULIFRONS*

Abstract

Morphologically discrete groups often exist within plant and animal species. Many social insect species possess dimorphic queens and workers whose distinct phenotypes likely evolved due to caste-specific selection. In this study, we investigated several facets of caste morphology in the social wasp *Vespula maculifrons* in order to broaden our understanding of caste-specific selection in social insects. Mass was the most dimorphic trait observed and also exhibited the lowest correlation between castes (intercaste phenotypic correlation). Caste dimorphism and intercaste phenotypic correlations for all measured traits were negatively associated. These trends are similar to those exhibited by sexually dimorphic phenotypes. Additionally, analyses of intracaste phenotypic correlations and trait size variation revealed that several queen traits (mass, thorax length, and thorax width) have experienced queen-specific selection. Finally, differences between worker and queen castes in the allometric relationships of mass, thorax length, and thorax width provide further evidence for the action of queen-specific selection.

Introduction

Within many species distinct groups often have different reproductive and functional roles, resulting in group-specific selective pressures (MAYNARD SMITH & BROWN 1984; ANDERSSON 1994; CHIPPINDALE *et al.* 2001). Group-specific selective regimes can produce morphological differentiation between groups, often modifying the genetic architecture underlying alternate phenotypes (LANDE 1980; 1987; BONDURIANSKY & ROWE 2005). When groups do not share the same phenotypic optima for homologous traits, selection is expected to favor dimorphism, with traits in each group evolving towards separate optima (LANDE 1980; FAIRBAIRN 1997; REEVE & FAIRBAIRN 2001). However, homologous traits are often controlled by the same genetic mechanisms resulting in intergroup genetic correlations (LANDE 1980; 1987). When intergroup genetic correlations for a homologous trait are high, selection acting on the trait in one group will cause a correlated response in the other (HARRISON 1953; KORKMAN 1957). Homologous traits that are highly correlated between groups and that undergo antagonistic group-specific selective regimes are sub-optimally expressed in both groups (CHIPPINDALE *et al.* 2001; BONDURIANSKY & ROWE 2005; BONDURIANSKY 2007a). When antagonistic constraints on fitness are diminished by a reduction in intergroup genetic correlations, specialized dimorphic phenotypes can evolve more rapidly (FISHER 1930; LANDE 1980; REEVE & FAIRBAIRN 1996; 2001; BONDURIANSKY & ROWE 2005; FAIRBAIRN & ROFF 2006; BONDURIANSKY & CHENOWETH 2009). For example, several sexually dimorphic species illustrate that highly dimorphic traits exhibit lower intersexual genetic correlations than traits that exhibit little dimorphism (ASHMAN 2003; BONDURIANSKY & ROWE 2005; FEDORKA *et al.* 2007; STEVEN *et al.* 2007).

Dimorphic groups have evolved in many social insects (ants, termites, some bees, and some wasps). In fact, the reproductive division of labor between queen and worker castes is one of the defining characteristics of social insects (WILSON 1971).

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Generally, queens are the primary reproductive females and are responsible for founding new colonies. In contrast, workers forage, maintain the nest, care for brood, and typically do not reproduce (HÖLLDOBLER & WILSON 1990). Caste-specific selective pressures, arising from drastically different life-histories, have produced morphologically distinct queens and workers in many social insects. Despite these morphological differences, female castes are generally determined by environmental, rather than genetic, factors (OSTER & WILSON 1978). Therefore, it is likely that homologous traits in reproductive and non-reproductive castes are highly genetically correlated and are controlled by the same genetic mechanisms. High intercaste genetic correlations in combination with caste-specific selection should result in the displacement of both castes from their phenotypic optima. Consequently, selection is expected to reduce intercaste genetic correlations for traits under strong caste-specific selection, giving rise to highly dimorphic castes that are near their phenotypic optima.

This study examines the effects of caste-specific selection on social insect morphology by analyzing correlations between and variation of queen and worker morphological traits in the social wasp *Vespula maculifrons*. Though caste dimorphism has been quantified in a wide variety of social insect species (for a review see BOURKE 1999), to our knowledge this is the first study to test whether the relationship between caste dimorphism and intercaste correlations follows the theoretical predictions and experimental evidence for dimorphic trait evolution in sexually dimorphic species (FISHER 1930; LANDE 1980; REEVE & FAIRBAIRN 1996; 2001; BONDURIANSKY & ROWE 2005; FAIRBAIRN & ROFF 2006; BONDURIANSKY & CHENOWETH 2009). In sexually dimorphic species, highly dimorphic phenotypic traits display low intersexual correlations. Therefore we predict a negative association between intercaste correlations and trait dimorphism in social insects.

Caste-specific selection not only affects trait size optima, but can also result in different relationships between traits (allometry; WILSON 1953; 1971; GOULD 1966; WHEELER 1991; BONDURIANSKY 2007b). Allometry describes the relationship between trait size and overall body size. The allometric relationship of a trait with overall body size indicates whether the size of a body part changes disproportionately with overall size (NIJHOUT & WHEELER 1996; TSCHINKEL 2008). Differences in allometries between groups are often linked to group-specific patterns of selection. Among social insects, specific allometric patterns often indicate functional differences both between queen and worker castes (HUNT *et al.* 1996), and among worker subcastes (WILSON 1953; OSTER & WILSON 1978; HÖLLDOBLER & WILSON 1990; DINIZ *et al.* 1994; FRASER *et al.* 2000; SCHONING *et al.* 2005). Traits experiencing different selective pressures in the two castes (i.e. those traits displaying pronounced caste dimorphism) are thus expected to show significant differences in their allometries between castes.

We investigated whether caste-specific selection has produced differences between *V. maculifrons* castes in their trait allometries. Though analyses of allometric relationships between reproductive and non-reproductive castes have been examined previously, prior studies have focused primarily on species that do not have morphologically discrete castes (JEANNE 1980; HARTFELDER & ENGELS 1992; JEANNE 1997; NOLL *et al.* 2004). Here we analyze allometric patterns in a social wasp with two phenotypically distinct female castes. Specifically, we determined which traits exhibit different allometric patterns between castes. Significant differences in allometries between castes would provide evidence for caste-specific selection in this species. Overall, our study broadens our understanding of caste and size evolution in a social wasp. Due to the high degree of female dimorphism in *V. maculifrons*, our study represents an important contrast to other studies of social insects as well as studies of sexual dimorphism.

Methods

Study Organism

We investigated caste dimorphism and the effects of caste-specific selection in the eastern yellowjacket *V. maculifrons*. This highly social wasp displays a high degree of dimorphism between its female castes. Queens are much larger than workers and differ from them in coloration, behavior, and physiology (BLACKITH 1958; SPRADBERY 1973; GREENE 1991). *Vespula maculifrons* castes are expected to be under distinct types of selection due to their strongly divergent morphologies, behaviors, reproductive roles, and functions (*V. maculifrons* natural history reviewed by SPRADBERY 1973; MACDONALD & MATTHEWS 1981; GREENE 1991). Additionally, *V. maculifrons* colonies produce abundant workers and new queens each year, thus providing the material necessary for morphological and genetic studies. Finally, the social structure displayed by *V. maculifrons* is well understood; colonies are headed by a single multiply mated queen (range in queen mate number of 3-9; ROSS 1985; GOODISMAN *et al.* 2007b) and, within a colony, members of all patrilines are found in both castes (GOODISMAN *et al*. 2007a). The presence of multiple full-sib and half-sib groups within colonies allows for the calculation of intercaste phenotypic correlations and permits analyses of allometric relationships both within and between colonies.

Samples

We collected 18 *V. maculifrons* nests in 2004 (*n* = 14) and 2005 (*n* = 4), from metropolitan Atlanta, Georgia (United States). Seventeen colonies contained only workers, 11 colonies contained both gynes (prereproductive queens) and workers, and only gynes were collected from one additional colony. A mean ($\bar{x} \pm$ SD) of 49.33 $\pm$ 35.02

Table 5.1: Sample sizes (n), means, standard deviations (SD), and coefficients of variation (CV) for five traits in *V. maculifrons* gynes and workers. The *t*-ratios (*t*) result from paired *t*-tests comparing worker and gyne CVs. Significant *t*-ratios indicate that the CVs differed between castes. Negative, or positive, *t*-values indicate that the CV of gynes was less than, or greater than, that of workers, respectively.

Trait	Year	n	Workers Mean ± SD	CV	n	Gynes Mean ± SD	CV	Between Castes *t*
Thorax Width	2004	705	2.30 ± 0.24	0.104	289	3.63 ± 0.19	0.053	-6.50**
	2005	155	2.32 ± 0.25	0.109	159	3.58 ± 0.15	0.043	-5.49*
Thorax Length	2004	705	3.64 ± 0.37	0.102	289	5.46 ± 0.33	0.061	-3.63*
	2005	157	3.69 ± 0.37	0.100	158	5.54 ± 0.34	0.066	-2.76
Third Tergum Length	2004	702	1.67 ± 0.22	0.133	289	2.84 ± 0.32	0.114	-0.60
	2005	157	1.67 ± 0.23	0.137	159	2.78 ± 0.39	0.141	1.63
Gaster Length	2004	690	5.70 ± 0.76	0.133	268	10.18 ± 1.34	0.132	0.52
	2005	153	5.45 ± 0.80	0.147	156	8.92 ± 1.14	0.128	-0.41
Mass	2004	723	14.36 ± 2.82	0.196	290	97.53 ± 23.51	0.241	3.77**
	2005	160	16.15 ± 4.08	0.253	160	70.98 ± 16.07	0.226	-0.80

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

workers and 37.58 ± 6.95 gynes per colony were used in our analyses (Table 5.1).

Genetic Analysis

      Vespula maculifrons queens are polyandrous. This results in the presence of multiple half-sib groups within a single colony. By assigning gynes and workers to patrilines within colonies using microsatellites, we were able to 1) calculate phenotypic correlations between full-sib gynes and workers (intercaste phenotypic correlations), and 2) determine whether genotype influenced allometric patterns.DNA was extracted from single legs of workers and gynes. Tubes containing leg samples were submerged into liquid nitrogen for 30 seconds. The legs were then immediately crushed using plastic pestles. Five hundred µl of 5% Chelex solution was added to the samples, which were then incubated at 95º C in a heat block for 20 minutes (WALSH *et al.* 1991).

      Worker genotypes were determined at eight microsatellite loci: LIST2003, LIST2004, LIST2013, LIST2019, LIST2020 (DALY *et al.* 2002), RUFA 5 (FOSTER et al., 2001), VMA-3, and VMA-6, (HASEGAWA & TAKAHASHI 2002). The heterozygosities at these loci were sufficiently high so that the probability of two males from the population having the same genotype (nondetection error; BOOMSMA 1996) was low ($<<$ 0.0001;GOODISMAN *et al.* 2007a). Gyne genotypes were determined at a subset of loci (LIST2004, LIST2013, LIST2019, LIST2020, and VMA-6), which were found to have sufficient genetic variation to distinguish all patrilines within colonies. PCRs were conducted as described by HOFFMAN *et al.* (2008), and PCR products were visualized using an Applied Biosystems PRISM® 3100 Genetic Analyzer. Gynes and workers were assigned to patrilines within colonies based on their multilocus genotype (JOHNSON *et al.* 2009).

Morphological measurements

After DNA extractions were completed, workers and gynes were dried overnight at 60° C in order to obtain their dry mass. Digital photos were then taken of all of the sampled workers and gynes. Each individual was photographed in a dorsally facing position. A Zeiss© millimeter micrometer was included in each photo to allow for calibration. Using tools within the Canvas 9.0.4 computer package, 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 length of the mesoscutum (TW), thorax length (TL), length of the third tergum (3TL), gaster length (GL; the measure of the abdomen not including the first abdominal tergum), and total body length (BL, used as the indicator of body size for bivariate allometric estimates, see below).

In this study, we present data for several different trait measurements allowing us to present a more complete analysis of dimorphism and allometry than the analysis of one or two traits would provide (TSCHINKEL *et al.* 2003; BONDURIANSKY & ROWE 2005). Some of the measured traits may be of functional importance in one or both of the castes. For example, mass and body length are related to survival and fecundity in a variety of social insects (HEINZE 1989; ELMES 1991; RÜPPELL *et al.* 1998; ABELL *et al.* 1999; DEHEER 2002; WIERNASZ & COLE 2003; BARGUM *et al.* 2004; FJERDINGSTAD 2005). Additionally, among social wasps, gaster structures often exhibit pronounced allometric caste differences (BLACKITH 1958; JEANNE & FAGEN 1974; TURILLAZZI *et al.* 1994). Recent studies suggest that *V. maculifrons* gyne length (i.e. GL and BL) is correlated with mating success as well as overwintering survival (KOVACS *et al.* 2008; unpublished). Consequently, several of these traits may be under different caste-specific selective

regimes and therefore allow us to test whether divergent selective pressures result in variation in trait dimorphism, intercaste correlations, or allometry.

Statistical Analyses

Caste Dimorphism

We determined the degree of trait dimorphism between workers and gynes using a modification of BONDURIANSKY and ROWE's (2005) formula originally derived to calculate the degree of sexual trait dimorphism relative to body size. The degree of caste dimorphism for each morphological trait *i* was thus calculated for each year separately as $D_i = 1 - (\overline{X}_i^W / \overline{X}_{BL}^W)/(\overline{X}_i^G / \overline{X}_{BL}^G)$; where $\overline{X}_i^j$ is the mean absolute size of trait *i* in caste *j* (*W* denotes worker and *G* denotes gyne caste), and $\overline{X}_{BL}^j$ is the mean total body length of caste *j*. This calculation provides an estimate of relative, rather than absolute, caste dimorphism. Relative trait sizes, because they include a measure of body length in their calculation, make it easier to interpret variation in body shape, as well as size (BONDURIANSKY & ROWE 2005)

Intercaste phenotypic correlations

We determined whether the mean worker trait size and the mean gyne trait size for full-sib groups within colonies were significantly correlated (intercaste phenotypic correlation; BONDURIANSKY & ROWE 2005). Such correlations would be expected if the genes workers and gynes inherited from their sires affected size variation similarly in the two castes (LANDE 1980). Due to the low trait size heritabilities previously estimated in this species (KOVACS *et al.* unpublished), we used intercaste phenotypic correlations instead of intercaste genetic correlations to test for a relationship between gyne and

worker trait size (CHEVERUD 1988; BONDURIANSKY & ROWE 2005). Spearman's rank correlations were used to test for phenotypic correlations between castes within each colony. The colony-level *P*-values for each independent Spearman's correlation were combined using a *Z*-transform test to determine overall significance for each year (WHITLOCK 2005). Additionally, the mean intercaste phenotypic correlations and the standard errors for each trait by year were calculated from colony estimates.

Morphological variation

　　We tested if workers and gynes differed in the amount of morphological variability they possessed by comparing the variation displayed by the seven measured traits in the two castes. Specifically, we calculated the coefficient of variation (CV) for each trait within the eleven colonies that contained both workers and gynes (FJERDINGSTAD & CROZIER 2006; ROUX *et al.* 2009). We then conducted paired *t*-tests for each year using caste-specific CVs to determine if worker traits displayed more variation than gyne traits.

Allometry

　　We began our investigation into the relationships between morphological traits by first calculating the Spearman's correlations (R_S) between traits for all individuals within each caste by year. We next investigated allometric relationships, measured by 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). A trait is isometric when *k* = 1.0, displays positive allometry when *k* > 1.0, and negative allometry when *k* < 1.0.

　　k values for each caste by year were calculated using the standardized loadings of the first eigenvector on all traits resulting from principal component analyses of the covariance matrix (JOLICOEUR 1963; GOULD 1966; SHEA 1985; DINIZ *et al.* 1994). By using principal component analyses to calculate the multivariate allometric coefficient

(*k*), overall body size was a product of a multivariate statistical method that more accurately describes size than any single characteristic would (HUMPHRIES *et al*. 1981; DINIZ *et al*. 1994). Means and standard errors for *k* were obtained by jackknifing over colonies. We then tested if *k* differed between the two castes, as well as between years, using a Student's *t* – test for each trait by year (SOKAL & ROHLF 1981; DINIZ *et al.* 1994).

We then determined whether *k* differed from isometry (*k* = 1.0) for each caste in each year (DINIZ *et al.* 1994; BONDURIANSKY 2007b). We note that, in contrast to linear traits, Mass is expected to increase geometrically with other linear measurements of size, because volume is a product of its three dimensions (i.e. the third power). Thus an isometric relationship between mass and overall body size would be denoted by *k* = 3 (WEST *et al.* 1997; TSCHINKEL 2008).

In addition, we tested whether allometric relationships varied between colonies and patrilines within colonies using bivariate regression methods (in contrast to the multivariate methods used to calculate *k* described above; FRASER *et al.* 2000). To conduct these analyses, we first used standardized major axis (SMA) regression to fit the log transformed trait value against log transformed total body length (BL) for individuals within each caste separately (DANFORTH & DESJARDINS 1999). We then ran three factor ANOVAs [year, colony (year), patriline (colony, year)] on the resulting individual residuals to determine whether allometric relationships differed significantly between years, colonies, or patrilines. Significant differences in residuals among levels (i.e. colony or patriline) would suggest significant differences in allometry for a trait at a given level (FRASER *et al.* 2000).

Results

Caste Dimorphism & Intercaste Phenotypic Correlations

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The sizes of most gyne and worker traits overlapped. That is, the largest workers

possessed traits equal in size to the smallest gynes (Figure 5.1). Mass was the only trait
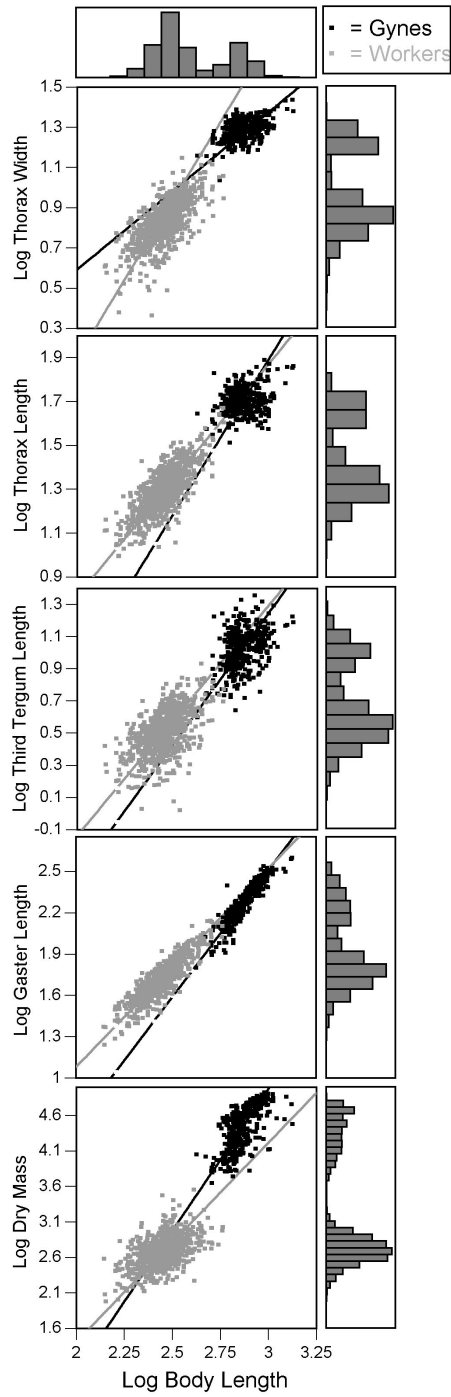
Figure 5.1: Static allometric relationships between five morphological traits and total body size in *Vespula maculifrons* workers and gynes (log transformed). Histograms for all traits in both castes are provided. Worker traits are represented by gray symbols and lines, while gyne traits are represented by black symbols and lines. All traits scaled linearly and positively to body size in both castes, though the allometric relationship between a trait and body size varied significantly between castes for nearly all measured traits (see text for details). Additionally, the histograms reveal that Mass is the only trait that displays no overlap between workers and gynes.

Table 5.2: Mean intercaste phenotypic correlations with standard error (SE) and significance (Spearman's correlations). Intercaste phenotypic correlations represent the correlation in trait size between full-sibling workers and gynes within colonies. Significant correlations ($P < 0.05$) are in bold. Caste dimorphism (CD) values reflect the degree dimorphism for particular traits. Negative, or positive, dimorphism values indicate that worker trait sizes were larger than, or smaller than, gyne trait sizes *relative* to total body length, respectively.

| Trait | Year | Intercaste Phenotypic Correlations | | Dimorphism |
		Mean Correlations ± SE	*P* - value	CD
Thorax Width	2004	0.105 ± 0.23	**0.03**	0.023
	2005	0.427 ± 0.18	0.41	0.050
Thorax Length	2004	0.314 ± 0.15	0.47	-0.028
	2005	-0.100 ± 0.13	0.91	0.024
Third Tergum	2004	-0.009 ± 0.19	0.74	0.094
Length	2005	0.282 ± 0.21	0.41	0.120
Gaster Length	2004	0.135 ± 0.19	0.52	0.137
	2005	0.264 ± 0.25	0.22	0.105
Mass	2004	-0.063 ± 0.15	0.99	0.773
	2005	-0.075 ± 0.17	0.68	0.667

that was completely discrete between the two castes. Mass also displayed the highest amount of dimorphism between the two castes in both years and consistently had the lowest intercaste phenotypic correlation (Table 5.2).

We investigated the relationship between caste dimorphism (the measure of trait size overlap in the two castes) and intercaste phenotypic correlation (the correlations of trait size between full sib workers and gynes) for our five traits. As predicted under a model where a positive intercaste correlation would constrain the evolution of dimorphism, the association between caste dimorphism and the intercaste correlations was negative in both years. However, neither of the trends in either year was statistically significant, (Figure 5.2; SMA regression; 2004: $y = -2.24x + 0.42$, $P = 0.19$; 2005: $y = -1.14x + 0.38$, $P = 0.40$).

Morphological Variation and Intracaste Correlations

There were significant differences between the castes in the amount of trait size variation exhibited by several traits (Table 5.1). Specifically, workers displayed significantly more variation in TW in both years and TL in one year, while gynes showed significantly more variation in Mass in one study year (Table 5.1). These differences in the amount of trait variation may be indicative of caste-specific selection acting on that trait.

All worker traits were strongly correlated with one another (Table 5.3, Spearman's correlations, $P < 0.0001$). While most gyne traits were also significantly correlated, several, most notably Mass and TL, were not (Table 5.3). Gyne Mass was uncorrelated with two other traits, TW and TL, and gyne GL and TL were uncorrelated in one study year (Table 5.3).
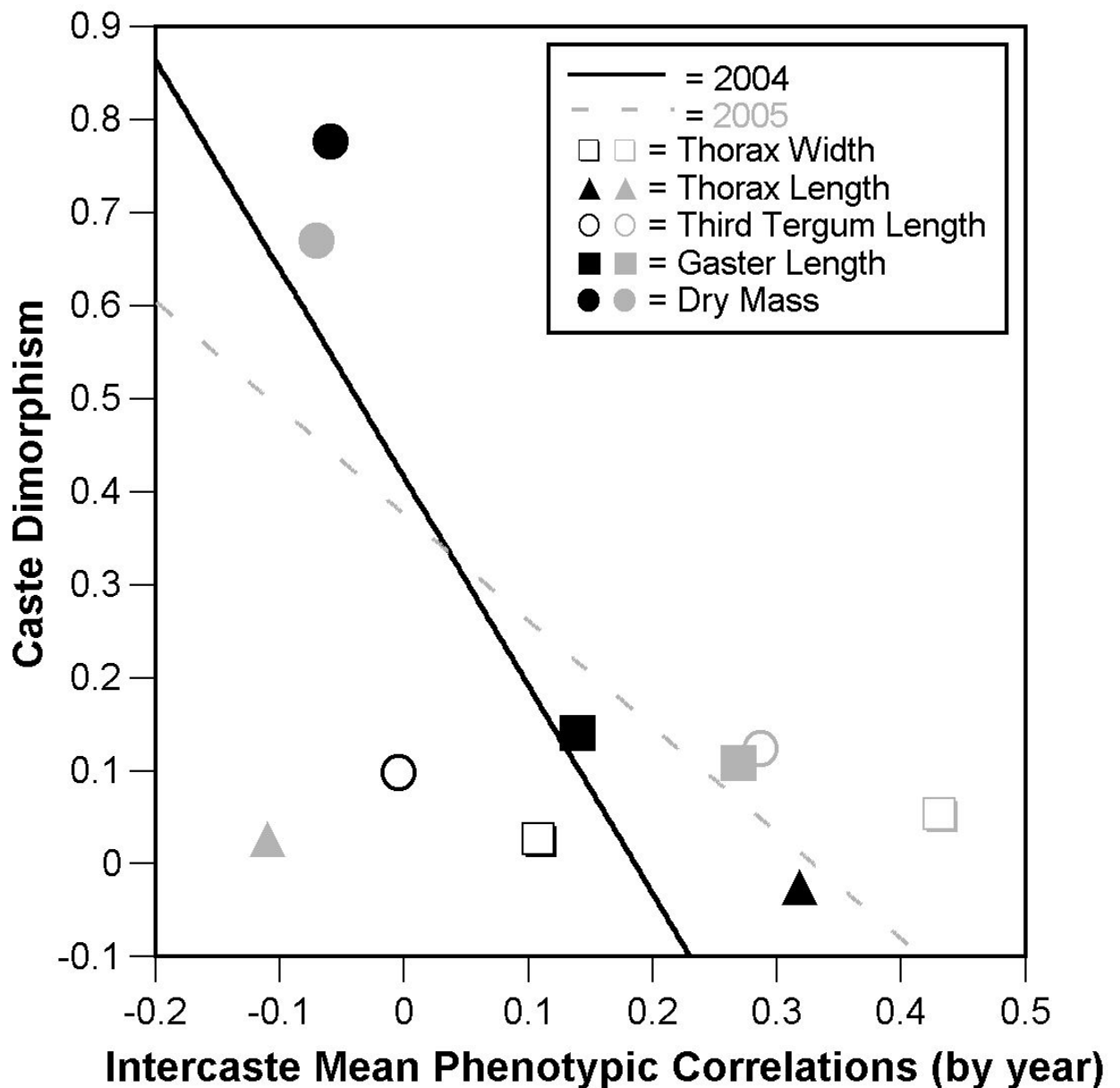
Figure 5.2: Degree of caste dimorphism for five morphological traits in *Vespula maculifrons* and their mean intercaste phenotypic correlations. Each morphological trait is represented by a unique symbol. All traits were measured in both 2004 and 2005. Black symbols and the solid black line were generated from 2004 samples, while gray symbols and the dotted gray line represent 2005 samples. Though neither the 2004 or 2005 regressions were statistically significant (SMA regression; 2004: *y* = -2.24*x* + 0.42 *P* = 0.19; 2005: *y* = -1.14*x* + 0.38, *P* = 0.40), they do reveal the negative trend expected under caste-specific selective regimes.

Table 5.3: Spearman's correlations (r^s) between five morphological traits in *Vespula maculifrons* workers and gynes. Gyne correlations lie above the diagonal; worker correlations lie below. Significant correlations ($P < 0.05$) are in bold. All worker traits are strongly correlated with each other ($P < 0.0001$), while some gyne traits are not significantly correlated with one another.

Trait	TW		TL		3TL		GL		Mass	
Year	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
TW			**0.46**	**0.33**	**0.38**	**0.22**	**0.31**	**0.25**	**0.14**	0.11
TL	**0.66**	**0.72**			0.09	**0.25**	0.08	**0.25**	-0.03	0.03
3TL	**0.42**	**0.59**					**0.49**	**0.48**	**0.37**	**0.19**
GL	**0.51**	**0.56**	**0.53**	**0.42**	**0.56**	**0.59**			**0.75**	**0.31**
Mass	**0.31**	**0.66**	**0.37**	**0.63**	**0.44**	**0.60**	**0.53**	**0.63**		

Table 5.4: Multivariate allometric coefficients (*k*) and first principal components for six morphological traits in *Vespula maculifrons* workers and gynes in two years. *t* ratios result from *t*-tests comparing *k* values from expectations under isometry (*k* = 1 for linear traits and *k* = 3 for Mass) in each caste. Significant negative *t* ratios indicate negative allometry, while significant positive *t* ratios indicate positive allometry. *t* – tests were also used to determine whether *k* estimates differed significantly between years for each caste. Negative *t* ratios resulted when the 2004 *k* estimates were greater than those in 2005. Additionally, Student's *t* –tests were used to test whether there were significant differences in *k* estimates between workers and gynes. Negative *t* ratios indicate that *k* estimates for gynes were greater than the *k* estimates calculated for workers, while positive *t* ratios represent the reverse.

Trait	Year	Workers PC1 ± SE	k ± SE	Isometry (t)	Years (t)	Gynes PC1 ± SE	k ± SE	Isometry (t)	Years (t)	Castes (t)
Thorax Width	2004	0.26 ± 0.009	0.63 ± 0.02	-73.20***	3.20*	0.03 ± 0.004	0.11 ± 0.01	-207.92***	1.43	79.99***
	2005	0.30 ± 0.03	0.74 ± 0.06	-8.37**		0.04 ± 0.01	0.13 ± 0.04	-49.47***		16.73***
Thorax Length	2004	0.27 ± 0.006	0.65 ± 0.01	-116.23***	-1.54	-0.01 ± 0.006	-0.02 ± 0.02	-153.27	4.26*	91.76***
	2005	0.24 ± 0.02	0.61 ± 0.06	-13.27**		0.04 ± 0.02	0.12 ± 0.06	-28.02***		11.22***
Third Tergum Length	2004	0.39 ± 0.007	0.95 ± 0.02	-10.82***	-1.97	0.18 ± 0.03	0.61 ± 0.007	-31.45***	2.63	25.28***
	2005	0.32 ± 0.06	0.80 ± 0.15	-2.67		0.26 ± 0.08	0.84 ± 0.17	-1.97		-0.31
Gaster Length	2004	0.43 ± 0.01	1.03 ± 0.03	4.01**	-1.25	0.35 ± 0.01	1.20 ± 0.03	19.75 ***	-1.98	-14.14***
	2005	0.38 ± 0.06	0.94 ± 0.13	-0.86		0.30 ± 0.10	0.96 ± 0.24	-0.32		-0.15
Mass	2004	0.72 ± 0.01	1.74 ± 0.03	-147.00***	7.11*	0.92 ± 0.007	3.10 ± 0.07	3.97**	-0.62	-51.12***
	2005	0.77 ± 0.01	1.92 ± 0.05	-46.78***		0.91 ± 0.05	2.95 ± 0.47	-0.21		-4.34*

* *P* < 0.05; ** *P* < 0.01; *** *P* < 0.0001

Allometry

The multivariate allometric coefficients k were significantly different between the castes for nearly all measured traits. (Table 5.4, $P < 0.05$). Only the allometric relationships for 3TL and GL were not significantly different between castes in both years. Thus, the relationship between trait sizes and overall body size differed significantly between castes for most traits. Though the k estimates were significantly different between the castes, the direction of allometry (i.e. positive or negative) tendedto be the same for most traits with the notable exception of Mass. Consequently, most allometric relationships in the two castes differed in their magnitude rather than their direction. Interestingly, while most worker and gyne traits displayed negative allometry, GL in both workers and gynes and Mass in gynes displayed positive allometry in one of the two years.

We detected significant differences between years, colonies, and patrilines in their bivariate allometric relationships for several traits (Table 5.5). Nearly all worker and gyne traits (except gyne 3TL) exhibited significant differences in their residuals at the colony level. In addition, the residuals for gyne TW and TL and worker GL differed significantly between patrilines within colony. Consequently, the allometric relationships displayed by castes depend on genotype as well as environmental effects.

Discussion

The goal of this study was to understand the effects of caste-specific selection on social insect morphologies. A trait may be under caste-specific selection if 1) it is dimorphic between the two castes, 2) it displays low intercaste phenotypic correlations, 3) there are significant differences in the amount of trait size variation present between the two castes, 4) intracaste phenotypic correlations with other morphological traits are low, or 5) the allometric relationship between the trait and overall body size differs significantly

Table 5.5: Results of three factor ANOVAs performed to test whether *V. maculifrons* worker and gyne traits varied significantly in their static allometric relationships between years, colony within year, and patriline within colony within year. Significant *P*-values are in bold. Significant differences between colonies and patrilines in trait allometries suggest that environment and genotype affect relationships between traits.

Trait	Factor	Workers			Gynes		
		F	*df*	*P*	*F*	*df*	*P*
Thorax Width	Year	1.11	1	0.29	3.13	1	0.08
	Colony (Year)	3.36	15	**0.0001**	2.45	9	**0.01**
	Patriline (Year, Colony)	0.94	79	0.63	1.46	58	**0.02**
Thorax Length	Year	6.01	1	**0.01**	2.68	1	0.10
	Colony (Year)	2.48	15	**0.002**	3.76	9	**0.0002**
	Patriline (Year, Colony)	1.25	79	0.08	1.70	58	**0.003**
Third Tergum Length	Year	6.08	1	**0.01**	0.07	1	0.79
	Colony (Year)	3.08	15	**0.0001**	1.39	9	0.19
	Patriline (Year, Colony)	1.24	79	0.09	0.95	58	0.58
Gaster Length	Year	0.002	1	0.97	0.29	1	0.59
	Colony (Year)	2.25	15	**0.004**	2.63	9	**0.006**
	Patriline (Year, Colony)	1.48	79	**0.007**	0.74	58	0.92
Mass	Year	0.37	1	0.54	2.99	1	0.09
	Colony (Year)	2.033	15	**0.01**	2.17	9	**0.02**
	Patriline (Year, Colony)	1.30	79	0.05	1.35	58	0.06

between castes. The combined results of our analyses are suggestive of caste-specific selection acting on several gyne traits, specifically mass, thorax width, and thorax length.

Caste Dimorphism and Intercaste Correlations:

Mass was the most dimorphic trait analyzed in this study, with all other traits displaying lower levels of dimorphism (Table 5.2, Figure 5.1). Previous studies quantifying dimorphism in *Vespula* wasps have found wing and gaster traits to be dimorphic between castes (BLACKITH 1958; JEANNE 1980). Generally, species belonging to the genus *Vespula* are recognized as having the most dimorphic castes within the vespine wasps (WILSON 1971). Indeed, the two castes can be clearly differentiated by their relative shape and size as well as coloration patterns. However, many of the morphological traits measured in this study showed overlap in size between the two castes, with some workers possessing traits that were as large as those found in the smallest gynes. Within *Vespula* colonies, workers are reared in smaller worker cells that are also used to rear males later in the season. In contrast, gynes are reared exclusively in larger queen cells that are produced only toward the end of the colony cycle. Therefore, the overlap in trait size between workers and gynes does not result from a shared rearing environment, but rather may be due to resource allocation in late season gyne-producing colonies, physiological constraints on body size due to functions such as flight, or other factors associated with caste evolution (see below).

Our analyses of caste dimorphism and intercaste correlations (i.e. trait correlations between full-sib workers and gynes) provide an intriguing parallel to analyses performed in sexually dimorphic species (ASHMAN 2003; BONDURIANSKY & ROWE 2005; FEDORKA *et al.* 2007; STEVEN *et al.* 2007). Caste dimorphism and intercaste correlations were negatively, though not significantly, correlated in both study years

(Figure 5.2). We do note that mass is more labile in adults than some other traits measured in this study, and therefore may be more readily influenced by environmental factors which may reduce intercaste correlations (MIRTH & RIDDIFORD 2007). Previous studies performed in sexually dimorphic species have found that highly dimorphic traits generally exhibit low phenotypic and/ or genetic correlations (ASHMAN 2003; BONDURIANSKY & ROWE 2005; FEDORKA *et al.* 2007; STEVEN *et al.* 2007). Negative relationships between dimorphism and intersexual correlations support the hypothesis that 1) the genetic architecture underlying sexual dimorphism is constrained by genetic correlations and 2) that dimorphism can more rapidly evolve if the intergroup correlations for homologous traits are reduced. This reduction in intergroup correlations is believed to allow both groups to evolve towards their optimal phenotypes more rapidly resulting in pronounced dimorphism (FISHER 1930; LANDE 1980; REEVE & FAIRBAIRN 1996; 2001; BONDURIANSKY & ROWE 2005; FAIRBAIRN & ROFF 2006; BONDURIANSKY & CHENOWETH 2009).

To our knowledge, this is the first study addressing the relationship between dimorphism and intergroup correlations performed in a caste dimorphic system. Our results suggest that both caste and sexually dimorphic traits evolve more rapidly when intergroup correlations are low. Further analyses performed in other caste dimorphic species are needed to more fully understand the genetic architecture of caste dimorphism, though our results do hint at the possibility that both caste-specific and sex-specific selection can alter the genetic architecture underlying dimorphism by reducing intergroup correlations.

Morphological Variation and Intracaste Phenotypic Correlations

Analyses quantifying 1) the amount of trait size variation expressed within a caste and 2) correlations between traits within individuals provide us with further

evidence of caste-specific selective pressures on several gyne traits. In general, traits that are under strong directional or stabilizing selection are expected to exhibit less size variation than traits that are under weak or diversifying selection (FALCONER & MACKAY 1996). We found that worker mass displayed less variation than gyne mass, while gyne thorax width and thorax length were significantly less variable than homologous worker traits (Table 5.1). Therefore, all three traits appear to be subject to caste-specific selective pressures.

Phenotypic correlations represent constraints on independent trait size evolution because correlations between phenotypic traits generally closely follow genetic correlations (CHEVERUD 1988; BONDURIANSKY & ROWE 2005). Much like the intergroup correlations discussed above, intracaste correlations among traits could result in sub-optimal phenotypes if selection operates differently on correlated traits. Therefore, antagonistic selective pressures may result in a reduction of intracaste phenotypic correlations. As a result, we would expect traits under caste-specific selection to be less correlated with other morphological traits than traits that are not under selection for multiple phenotypic optima. We found that all worker morphological traits were highly correlated with one another, while several sets of gyne traits were uncorrelated. Specifically, gyne mass and thorax length; mass and thorax width; and gaster length and thorax length were not significantly correlated (Table 5.3).

Taken together these analyses of trait variability and trait intracaste correlations suggest that thorax width, thorax length, and mass are under stronger selection in gynes than workers. Based solely on the levels of variation in mass present in the two castes, it would seem that mass is under stronger selection in workers relative to gynes, since gyne mass displays significantly more variability than worker mass. However, this conclusion is not supported by gyne and worker intracaste phenotypic correlations. The strong correlations found between all worker traits, including mass, are not consistent

with selection on mass in workers. Rather, the non-significant correlations found between gyne mass and gyne thorax width and thorax length indicate that significant differences in the amount of worker and gyne mass variation are more likely the result of selective pressures on gyne mass than worker mass. This discrepancy may be explained by mass being uncorrelated with other morphological traits simply due to its ability to change over an individual's lifetime. Additionally, the presence of a third set of uncorrelated gyne traits, thorax length and gaster length, indicates that gyne thorax traits may also be under gyne-specific selection. Overall, these findings suggest the action of caste-specific selection on gyne thorax width and thorax length, and perhaps mass in *V. maculifrons*.

Allometry

Caste-specific selection can produce changes not only in a trait's size, but also in its relationship with overall body size (allometry). We found that most traits in both workers and gynes displayed negative allometry, with the exception of worker and gyne gaster length and gyne mass. Negative allometric relationships indicate that traits are disproportionately small in relation to overall body size (Table 5.4). Unfortunately, allometry estimates from previous studies are difficult to compare due to a lack of compatibility of trait measurements and analyses. Additionally, few allometric relationships, even for nearly identical measure traits, seem to be consistent across species (NOLL *et al*. 2004). Previous work (BLACKITH 1958), in the closely related species *Vespula rufa, V. germanica, and V. vulgaris,* found that gyne gaster traits were positively allometric, which is consistent with our results in one study year (Table 5.4).

In this study, the greatest differences between worker and queen allometries were displayed by thorax width, thorax length, and mass (Table 5.4). A number of studies examining caste allometries in social vespid wasps have identified shape

differences between reproductive and non-reproductive individuals, even in species without morphologically discrete castes (BLACKITH 1958; JEANNE & FAGEN 1974; HUNT *et al.* 1996; NOLL *et al.* 2004; NOLL & WENZEL 2008). Any differences in the allometric relationships of hard body parts present in the adult stage of holometabolous insects are the result of growth differences during development (STERN & EMLEN 1999; SHINGLETON *et al.* 2007). This is because, in holometabolous insects, the growth of hard-body morphological traits is restricted to the embryonic and larval growth stages (MIRTH & RIDDIFORD 2007). Pre-imaginal caste determination is well-documented in *V. maculifrons,* and so we expect allometric differences between the castes for some traits. However, the strong allometric differences detected between the castes for all observed traits, in combination with the overlap between castes in most trait sizes, indicates that caste evolution in *Vespula* wasps may have followed a model proposed by WHEELER (1991). Wheeler's model of caste evolution begins with casteless individuals that have the same allometric relationships regardless of body size. Castes begin to form when different size groups occur and subsequently diverge according to selection on 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 than in size.

We were interested not only in determining whether trait allometries differed between castes, but also in whether a trait's allometric scaling relationship differed between 1) years, 2) colonies (environment), and 3) patriline within colony (genotype). Interestingly, while few differences occurred between years, worker and gyne traits did show significant differences between colonies in their scaling relationships with body size (with the exception of gyne third tergum length). There were also significant

differences between patrilines within colony in the allometries of gyne thorax width and thorax length as well as worker gaster length (Table 5.5).

The differences in static allometries at the colony and patriline level observed in this study are due to environmental and, for some traits, genetic influences on growth. Studies performed in both social and non-social insects examining static allometries in different environments and genetic backgrounds have found significant influences of both factors on allometric scaling relationships (FRASER *et al.* 2000; GILCHRIST & PARTRIDGE 2001; OWEN & HARDER 2002; FUKUDA *et al.* 2003). The large number of traits, both in workers and gynes, that possess environmentally variable allometric scaling relationships is surprising. However, environmentally sensitive allometries are an important example of the phenotypically plastic nature of social insects.

Conclusions

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

CHAPTER 6

CONCLUSIONS

My dissertation encompasses four studies examining caste dimorphism and the caste-specific evolutionary pressures that have shaped worker and queen phenotype in the social wasp *Vespula maculifrons*. My work has examined queen morphological traits associated with mating success and overwintering success (two fitness related traits), as well as the genetic architecture underlying caste dimorphism in workers and queens. Overall, my findings suggest that queen-specific selective pressures have played an important role in the evolution of social insect caste dimorphism.

Queen gaster length is associated with mating success and overwintering

survival: An effect of female activity or male mate choice?

Chapters 2 and 3 sought to identify queen traits associated with increased queen mating success and overwintering survival. Several queen traits were positively associated with both queen mating success and overwintering survival in multiple years; of this suite of traits, gaster length was consistently associated with both fitness traits in all three years. The association between gaster length and mating success can potentially be explained by two non-mutually exclusive theories. In both theories, queen gaster length is strongly associated with overwintering survival and therefore female fitness.

In the first theory, queen mating success is simply a function of queen activity. More active queens also possess longer gasters than less active queens, and therefore the association between mating success and gaster length are the result of queen, rather than male, activity. Female size, particularly mass, is positively associated with

queen productivity and survival in a number of ant species (VANDER-MEER *et al.* 1992; BERNASCONI & KELLER 1999; WAGNER & GORDON 1999; DEHEER 2002; TSCHINKEL *et al.* 2003; WIERNASZ & COLE 2003; FJERDINGSTAD 2005), as well as other insects (for a review see BONDURIANSKY 2001). Under this scenario, the data presented in chapters 2 and 3 can be interpreted as follows; higher fitness queens are often longer and more active. Due to this increased queen activity, these higher fitness queens mate at higher frequencies than lower fitness queens.

The results in chapters 2 and 3 can also be interpreted in another, perhaps complimentary fashion. In the second theory, males use queen gaster length as an indicator of female fitness and are therefore preferentially mating with queens with long gasters. Males are predicted to demonstrate mate choice when 1) males allocate valuable resources to mating, 2) when female fitness is highly variable, and 3) the cost of mate search and assessment is low (BONDURIANSKY 2001).

The conditions for male mate choice appear to be met in *V. maculifrons,* as well as other social insects. For instance, among the hymenoptera, sperm is a valuable resource since males are sperm-limited due to testes degeneration during larval development (HÖLLDOBLER & BARTZ 1985). Additionally, queens vary drastically in their fitness due to the high levels of mortality experienced during a colony cycle (SPRADBERY 1973; HÖLLDOBLER & WILSON 1990). Finally, though numerical sex ratios for most social insects are male-biased (PAMILO 1982), the natural mating behavior and mate-search strategies for *V. maculifrons* are largely unknown (SPRADBERRY 1973), making it difficult to ascertain the cost of mate search in this species. Overall, what we do know about the physiology and life-history of *V. maculifrons* reproductives, suggests that males may prefer to mate with longer, higher fitness females, as has been reported in a number of insect and animal species (OWENS & THOMPSON 1994; JOHNSTONE *et al.* 1996; JOHNSTONE 1997; BONDURIANSKY 2001; BATEMAN & FLEMING 2006).

Though both an effect of queen activity and male mate choice are consistent with our observations of queen mating success in *V. maculifrons*, definitively separating these two theories experimentally would be a difficult endeavor. For instance, while female mate choice generally operates on male ornaments and other secondary sexual characteristics, most studies investigating male mate choice have identified preferences for female size traits, such as mass, which are also associated with female fecundity and survival (BONDURIANSKY 2001; DOSEN & MONTGOMERIE 2004; but see HUNT *et al.* 1999; AMUNDSEN & FORSGREN 2001). This link between sexually selected and naturally selected female traits presents a perhaps insurmountable problem to experimental manipulation of male mate choice in most systems (but see JONES *et al.* 2001; CHENOWETH *et al.* 2007). For example, in *V. maculifrons*, experimentally manipulating queen length, while not affecting queen quality or behavior, would be extremely difficult, and since males are expected to prefer higher quality queens such manipulations may prove largely uninformative.

In addition to identifying morphological traits associated with queen mating success, we uncovered a genetic component to mating success. Specifically, queens from particular patrilines mated at higher frequencies than those belonging to other patrilines. This effect of genotype on queen mating success was surprising, especially since it appears to be largely independent of queen length and overwintering survival. Therefore, genotype may be associated with another trait not measured in this study. For example, there may be an effect of genotype on pheromone composition, the size of another morphological trait, or a component of female fitness other than overwintering survival. Alternatively, genotype itself may be associated with queen mating success due to genotype-specific male-female interactions (ZEH & ZEH 1997; SCHWANDER & KELLER 2008).

Whatever the underlying cause, the identification of two independent traits, genotype and gaster length, associated with increased mating success may shed light on the variable relationship between gaster length and mating success across years, as well as support the male mate choice hypothesis. Often when multiple cues are used to assess mate "attractiveness", there is no one universally attractive phenotype, and mate preference is variable (JENNIONS & PETRIE 1997; WIDEMO & SAETHER 1999; MURPHY & GERHARDT 2000; BROOKS & ENDLER 2001; CANDOLIN 2003) Therefore the identification of two possible independent cues, as well as variation in gaster length preference between years, is suggestive of male mating preferences based on several queen cues, only one of which is gaster length.

In summary, chapters 2 and 3 sought to identify queen traits associated with queen fitness in *V. maculifrons*. Queen gaster length was associated with mating success in all study years, though not always positively. Additionally, queen gaster length was positively associated with increased overwintering survival. Based on these findings, it appears that queen gaster length is under natural and/or sexual selection in *V. maculifrons*, depending on whether or not male mate choice is operating in this species. Therefore these studies provide evidence for a functional role of queen gaster length, thereby laying the groundwork for further analyses identifying selection on worker and queen morphological traits.

Low amounts of genetic variation for most worker and queen traits, except queen gaster length: A role for non-additive and indirect genetic effects?

In Chapter 4, I used classical quantitative genetic techniques to analyze levels of genetic variation underlying morphological trait size in *V. maculifrons* workers and queens. I began by calculating the broad and narrow sense heritabilities for several worker and gyne morphological traits. Estimates of heritability provide information on the

amount of genetic variation underlying phenotypic variation and therefore can be used

as indicators of the selective pressures that have acted on traits (ROFF 1997). For

example, strong, multi-generational directional selection is predicted to decrease the

amount of additive genetic variance in a population resulting in low heritability

(FALCONER & MACKAY 1996). Therefore, I predicted that queens would display lower

heritability for size than workers, particularly for queen gaster length, a trait associated

with mating success and overwintering survival.

Though very little genetic variation was detected for most worker and queen

traits, broad sense heritabilities were significant for queen thorax width, gaster length,

body length, and mass. The existence of genetic variation for traits potentially linked to

fitness components, is somewhat surprising. This is especially true for gaster length, a

trait predicted to be under selection, because directional selection is expected to remove

additive genetic variation for that trait from the population (MOUSSEAU & ROFF 1987;

FALCONER 1989). However, recent theoretical work suggests that the influence of non-

additive and indirect genetic effects make the relationship between heritability estimates

and selection not as straight-forward as previously assumed (WOLF *et al.* 1998; MERILA

& SHELDON 1999).

Non-additive genetic effects, specifically dominance and epistatic interactions,

may affect heritability estimates for traits linked to variation in fitness, such as queen

gaster length. If directional selection quickly erodes additive genetic variation for a

fitness trait, then most of the remaining genetic variation would be due to dominance

effects. Therefore the contribution of dominance is expected to be greater in fitness traits

than non-fitness traits (ROFF *et al.* 1997; MERILA & SHELDON 1999;). For our work in *V.*

maculifrons, all significant queen heritabilities were broad sense estimates, which would

introduce a dominance variance component into our calculations. Additionally, some

studies have revealed that fitness-related traits are often controlled by more loci than

non-fitness related traits (FALCONER 1989; ARMBRUSTER *et al.* 1997) and therefore epistatic interactions can also substantially contribute to variation in fitness-related traits (LYNCH & WALSH 1998).

Indirect genetic effects may also influence heritability for fitness-related traits, especially in social insects. When the social environment, such as a colony, affects the expression of a trait, genes expressed in social partners (indirect genetic effects) as well as an individual's own genes (direct genetic effects) affect a phenotype (WADE 2001; LINKSVAYER & WADE 2005; LINKSVAYER 2006). Therefore, phenotypes are shaped by the genotypes of multiple interacting individuals (WILHAM 1963; CHEVERUD 1984; LYNCH 1987; CHEVERUD & MOORE 1994; MOORE *et al.* 1997). In social insects, genes expressed in queens affect offspring traits indirectly by influencing maternal performance, as do genes expressed in care-giving workers through sibsocial performance (LINKSVAYER & WADE 2005; LINKSVAYER 2006). Unfortunately, few studies have examined maternal and sibsocial effects in social insects (BIENEFELD & PIRCHNER 1990; LINKSVAYER 2006). Those that have quantified these effects have found that both direct and indirect genetic components can have a large effect on phenotype as well as overall heritability estimates (LINKSVAYER 2006). In the heritability analyses presented in Chapter 4, I was unable to tease apart direct, maternal, and sibsocial effects on offspring phenotype. Though for nearly every measured trait in both years, the proportion of phenotypic variation explained by colony was larger than that explained by genotype (patriline). Therefore in *V. maculifrons,* as in other social insects, the colony environment (i.e. the social environment) has a large impact on individual phenotypes, and therefore indirect genetic effects may contribute to the significant heritability estimates calculated for some fitness-related morphological traits.

Queen thorax length is under queen-specific selection, but gaster length is not:

Can these results be reconciled?

In Chapter 5, I identified several morphological traits that appear to be under queen-specific selection based upon 1) their low intercaste phenotypic correlations, 2) pronounced dimorphism, 3) differences between castes in the amount of trait size variation, 4) their low intracaste phenotypic correlations, or 5) differences in allometric relationships between castes. These analyses suggest that gyne thorax width, thorax length, and mass are under queen-specific selection. It is interesting to note that these queen traits were not previously identified as being associated with overwintering survival or mating success. Based on the results of the mating and overwintering trials presented in chapters 2 and 3, gaster length would be predicted to display morphological patterns consistent with queen-specific selection. However, gaster length did not appear to be under queen-specific selection in these additional analyses.

I would like to suggest a possible interpretation that could reconcile the data from all four studies as well as outline how queen-specific selection has shaped queen length, particularly thorax and gaster length, in *V. maculifrons*. Contrary to my original predictions, queen gaster length did not appear to be under queen-specific selection, while queen thorax length did. This finding can be interpreted as representing a trade-off between queen thorax and gaster length relative to overall body length. The results of several analyses presented in chapter 5 provide evidence for the inverse relationship between thorax and gaster length in *V. maculifrons* queens. Firstly, queen thorax length and gaster length are both strongly correlated with body length, indicating they are the two major measured components of body length. Additionally the multivariate allometric analyses revealed that thorax length was negatively allometric, while gaster length displayed either positive allometry or isometry. This means that thorax length is disproportionately small in relation to overall body size, while gaster length is

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disproportionately large or at least equal in proportion to overall body size. So, as thorax length gets smaller, gaster length gets bigger in relation to overall body size. Therefore, the relationship between queen thorax length and gaster length can be interpreted as a trade-off, with thorax length being smaller than gaster length in proportion to total body length.

Further evidence for the negative association between gaster and thorax length relative to overall body size comes from hymenopteran developmental patterns. Hymenopteran insects are holometabolous and therefore do not undergo molting as adults. This means that the size of hard body parts is determined during larval development (NIJHOUT & WHEELER 1996). Because hard body trait sizes, such as thorax length, are fixed prior to adulthood, they may be under stricter genetic control than traits that are malleable during adulthood, such as gaster length. If it is easier to detect evidence of selective pressures on hard body parts due to their "fixed" nature, then queen-specific selection on thorax length may be suggestive of a negatively correlated evolutionary response between gaster length and thorax length in relation to total body length. Though none of our morphological analyses revealed the action of queen-specific selection on gaster length itself, the selective pressures that effect thorax length may be indirectly affecting gaster length, and through this correlated response, selection is favoring queens with smaller thoraxes and therefore longer gasters in proportion to total body length. Therefore, if a trade-off between gaster and thorax length is present in this species, then the evidence for queen-specific selection on thorax length would be consistent with the association between gaster length and fitness observed in chapters 2 and 3.

Conclusions

Social insects are the most abundant animals in most terrestrial ecosystems (WILSON 1971). A key component to their ecological dominance has been the reproductive division of labor between workers and queens. In many social insects, reproductive queens mate, hibernate, found colonies, and are the primary egg-layers within colonies, while sterile workers forage and are responsible for nest-building and brood care (WILSON 1971). In addition to their distinct functional and reproductive roles, workers and queens often exhibit pronounced morphological differences which can further enable task specialization. Due to the importance of caste and caste dimorphism to the evolutionary and ecological success of social insects, it is fundamental to understand how these dimorphic groups evolved as well as the modern evolutionary pressures that continue to shape them.

In this dissertation, I have presented four studies that sought to elucidate the selective pressures that have affected caste morphologies as well as the genetic architecture underlying caste-specific phenotypes. These studies identified queen morphological traits that appear to be under queen-specific selective pressures due to selection events that are not experienced by workers. These results suggest that queen-specific selective pressures associated with overwintering survival and mating success play an important role in the evolution of caste dimorphism. In the broader context of social insect evolution, my results highlight the importance of selective pressures associated with life-history events, such as mating, overwintering, colony founding, and egg-laying, in shaping both caste morphology and the genetic architecture underlying phenotype in highly social systems.

Future Directions

Several possible paths of study can be followed using the work presented here as a base. One, which is currently being analyzed, examines the morphological differences between gynes (pre-reproductive queens) and foundress queens over multiple years. There appear to be significant differences in gaster length, thorax length, and body length between gynes in different years. Thorax length is consistently smaller and body and gaster length is consistently larger in subsequent years. This finding is consistent with the results presented in this dissertation and is suggestive of a trade-off between thorax length and gaster length in gynes. Interestingly, this same pattern is not observed in foundress queens, though when data across all years are combined, foundress queens have significantly shorter thoraces and longer gasters than gynes. This result suggests that successful queens possess gaster and thoraces within a limited size range and that gynes with trait sizes outside those ranges are not as fit and therefore do not produce successful colonies.

Several additional studies could also be performed in order to better understand the mating behavior of *V. maculifrons*. In chapters 2 and 3, several queen morphological traits were associated with mating success, which is suggestive of pre-copulatory male mate choice. Males may also assess queen quality during mating which may affect the number of sperm a male transfers to a female during mating (i.e. cryptic male choice). To determine whether this is indeed occurring in this system, sperm counts of the spermethecal contents of queens used in mating trials could be performed to determine whether queen morphological traits are associated with the number of sperm a queen receives during mating. Additionally, male body size may be associated with the number of sperm he possesses and therefore male body size could play a role in determining the number of matings a male could participate in due to sperm depletion.

In chapter 3, we detected an effect of genotype on mating success with individuals of some patrilines mating at higher frequencies than others. One explanation for this observation is that there is a genotypic component to pheromone composition. To determine whether cuticular hydrocarbons (which can operate as pheromones) vary between patrilines within the same colony, an experiment could be designed in which the composition of cuticular hydrocarbons are determined for individuals from multiple patrilines and multiple colonies. We could then assess whether genotype plays a significant role in determining an individual's cuticular hydrocarbon composition, which may explain the patriline effect on mating success that was observed.

Finally, in order to more fully understand dimorphism in social wasps, similar morphological analyses to those performed in chapters 4 and 5 could be done on male morphology. This would allow for a more thorough analysis of both social insect caste and sex dimorphism. We could then identify selection patterns that are male- and female-specific as well as caste-specific. Overall, the studies presented here provide the experimental and theoretical bases for future studies in multiple different areas of interest, including mating behavior and mate choice, as well as morphometrics and chemical ecology.

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