

# Mating Success in the Polyandrous Social Wasp *Vespula maculifrons*

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## 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 has on colony

structure and diversity (Crozier & Fjerdingstad 2001; Strassmann 2001).

Social insect 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).

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) female quality is highly variable, 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 the 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 because of 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. *Vespula maculifrons* is found in the eastern USA and builds large, annual, subterranean nests (MacDonald & Matthews 1981; Greene 1991). *Vespula 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, although 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,b; 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) whether large size was associated with male mating success, as is the case in other animal taxa (Blanckenhorn 2005); (2) whether individuals avoided mating with their nestmates, which is expected if consanguineous matings result in low fitness (Zeh & Zeh 2003); and (3) whether 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.

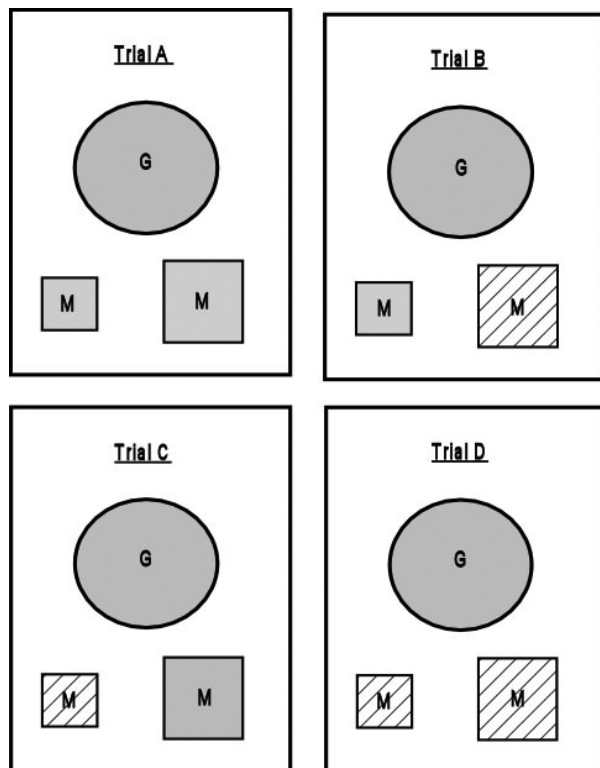
## Methods

### Collection and Maintenance of Samples

Six underground *V. maculifrons* colonies were collected from locations around Atlanta, Georgia, USA, between Sep. and Oct. 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 45-min trial, one female was presented with two males belonging to different size classes (large vs. small). Additionally, mating trials were set up such that (1) the males could both be from the same colony as the gyne, (2) the males could both be from different colonies than the gyne, or (3) one male could be from the same colony as the gyne and the other male 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. 1). The two males were marked with different colored dots of paint on their thoraces. Our pre-trial experimentation indicated that these small paint dots,



**Fig. 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 *Vespula 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 the 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 than the gyne. G, gyne; M, male.

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.

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<sup>®</sup> millimeter micrometer (Carl Zeiss Microimaging, Thornwood, NY, USA) 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 (ACD Systems, Victoria, BC, Canada).

## 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 traits for both males and gynes. All statistical analyses were conducted using JMP 3.2.6 (SAS Institute, Cary, NC, USA).

## Mating Success and Size

We determined whether the 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 the 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 whether 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 that 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 chi-square 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 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 the trait size.

The values for most pairs of male traits were significantly ( $\alpha = 0.05$ ) correlated, with the exceptions of TW–TL and TW–T3L (Table 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 approx. 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 1). Surprisingly, many of the traits in gynes

**Table 1:** Means and standard deviations ( $\bar{x} \pm \text{SD}$ ) of nine size metrics for *Vespula maculifrons* males and gynes by colony (linear size measurements in millimeters and mass in milligrams)

Trait	Colony-of-origin					
	A	B	C	D	E	F
<i>Males</i>						
Thorax width (TW)*	–	–	2.73 <sup>2</sup> ± 0.09	3.11 <sup>1</sup> ± 0.08	2.55 <sup>2,3</sup> ± 0.08	2.33 <sup>3</sup> ± 0.07
Thorax length (TL)*	–	–	3.40 <sup>2</sup> ± 0.09	3.76 <sup>1</sup> ± 0.07	3.78 <sup>1</sup> ± 0.08	3.67 <sup>1,2</sup> ± 0.07
Head to end of thorax length (HTL)*	–	–	4.66 <sup>3</sup> ± 0.09	5.30 <sup>1</sup> ± 0.07	5.00 <sup>2</sup> ± 0.08	4.75 <sup>2,3</sup> ± 0.07
Tergum 3 length (T3L)*	–	–	1.54 <sup>2</sup> ± 0.04	1.83 <sup>1</sup> ± 0.04	1.61 <sup>2</sup> ± 0.04	1.61 <sup>2</sup> ± 0.04
Gaster length (GL)*	–	–	8.10 <sup>2,3</sup> ± 0.17	8.86 <sup>1</sup> ± 0.14	8.27 <sup>2</sup> ± 0.15	7.66 <sup>3</sup> ± 0.13
Antennal length (AL)*	–	–	7.76 <sup>2,3</sup> ± 0.13	8.00 <sup>1</sup> ± 0.10	7.96 <sup>1,2</sup> ± 0.13	7.52 <sup>3</sup> ± 0.12
Total body length (BL)*	–	–	13.15 <sup>1,2</sup> ± 0.23	14.59 <sup>1</sup> ± 0.19	13.76 <sup>1,2</sup> ± 0.20	13.05 <sup>2</sup> ± 0.18
Distal cell length (CL)*	–	–	5.23 <sup>2,3</sup> ± 0.07	5.58 <sup>1</sup> ± 0.06	5.38 <sup>1,2</sup> ± 0.07	5.09 <sup>3</sup> ± 0.06
Mass*	–	–	19.19 <sup>2</sup> ± 1.12	24.27 <sup>1</sup> ± 0.89	17.41 <sup>2</sup> ± 0.95	16.21 <sup>2</sup> ± 0.85
<i>Gynes</i>						
TW*	3.67 <sup>1</sup> ± 0.05	3.50 <sup>1,2</sup> ± 0.07	3.42 <sup>2</sup> ± 0.06	3.62 <sup>1,2</sup> ± 0.05	3.48 <sup>2</sup> ± 0.04	–
TL*	5.32 <sup>1,2</sup> ± 0.08	4.91 <sup>2,3</sup> ± 0.13	5.16 <sup>1,2,3</sup> ± 0.10	5.37 <sup>1</sup> ± 0.08	5.00 <sup>3</sup> ± 0.06	–
HTL*	7.00 <sup>1</sup> ± 0.09	6.53 <sup>1,2</sup> ± 0.14	6.75 <sup>1,2</sup> ± 0.11	6.98 <sup>1</sup> ± 0.09	6.58 <sup>2</sup> ± 0.07	–
T3L*	2.56 <sup>1</sup> ± 0.06	2.18 <sup>2,3</sup> ± 0.10	2.43 <sup>1,2</sup> ± 0.08	2.44 <sup>1,2</sup> ± 0.07	2.17 <sup>3</sup> ± 0.05	–
GL*	8.62 <sup>1,2</sup> ± 0.24	7.52 <sup>2,3</sup> ± 0.37	8.75 <sup>1,2</sup> ± 0.29	8.90 <sup>1</sup> ± 0.25	7.51 <sup>3</sup> ± 0.18	–
AL	5.17 ± 0.08	5.04 ± 0.11	5.03 ± 0.08	5.22 ± 0.07	4.99 ± 0.05	–
BL*	16.69 <sup>1</sup> ± 0.25	15.20 <sup>2,3</sup> ± 0.38	16.44 <sup>1,2</sup> ± 0.30	16.79 <sup>1</sup> ± 0.26	15.30 <sup>3</sup> ± 0.19	–
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	–

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.

**Table 2:** Spearman's correlation coefficients ( $r_s$ ) between size metrics for *Vespula maculifrons* gynes (above the diagonal) and males (below the diagonal)

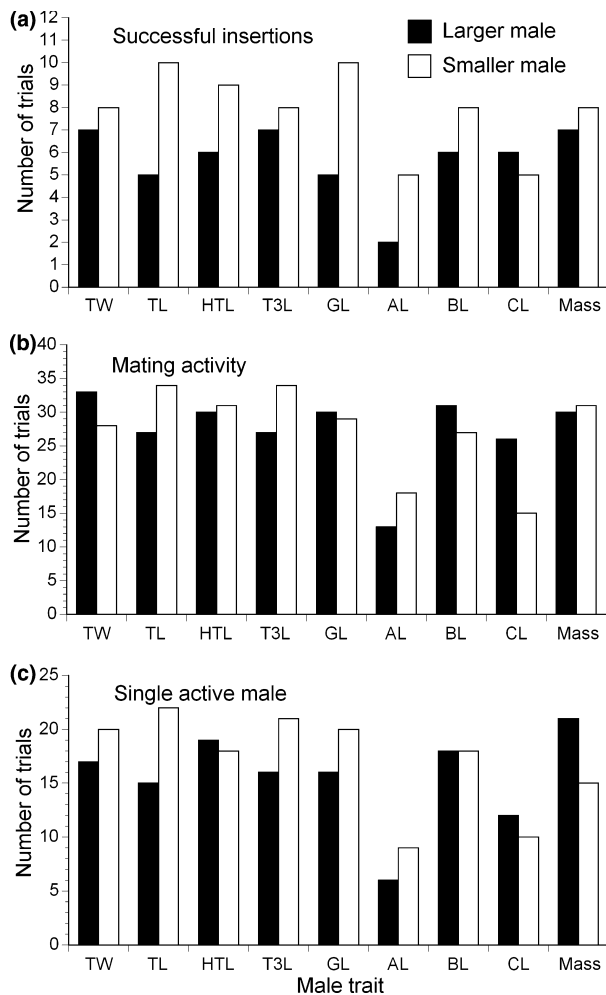
Trait	TW	TL	HTL	T3L	GL	AL	BL	CL	Mass
TW	–	<b>0.432</b>	<b>0.462</b>	0.243	0.224	<b>0.560</b>	<b>0.359</b>	0.070	0.134
TL	0.026	–	<b>0.809</b>	<b>0.494</b>	<b>0.546</b>	<b>0.498</b>	<b>0.701</b>	0.203	0.332
HTL	<b>0.400</b>	<b>0.674</b>	–	<b>0.341</b>	<b>0.445</b>	<b>0.498</b>	<b>0.694</b>	0.096	0.210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

Statistically significant correlations are shown in **bold** ( $\alpha = 0.05$ ). See Table 1 for trait abbreviations.

were uncorrelated. In total, 14 of the 36 Spearman's correlation coefficients were not significant (Table 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 the general body size. PC2 accounted for 15% of the total variance and was positively correlated with TW, HTL, TL, and AL.

### Mating Success and Size

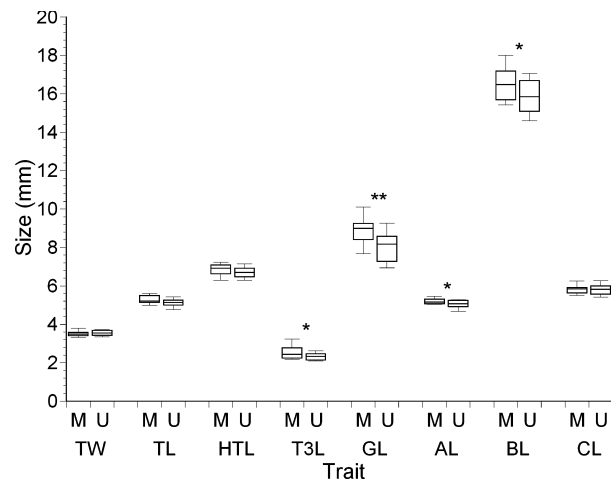
Of the 90 trials conducted, 67 resulted in some mating activity (mounting or insertion). Mounting time of males ranged from 12 s to 1830 s. A total of 26 matings were observed in 15 different trials. Few male–male interactions were observed during the trials; in some cases, a male would attempt to mount a male that was either mounting the gyne or



**Fig. 2:** Relationship between male mating behavior and male trait size in the social wasp *Vespula 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).

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 15 trials in which insertions were observed,



**Fig. 3:** Box plots illustrating the size of linearly measured traits in mated (M) and unmated (U) *Vespula 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).

there was no significant difference in mating success between males possessing smaller or larger traits (Fig. 2A; sign test:  $p > 0.05$ ; power analyses were run for data subsets; for a subset of 15 trials and  $\alpha = 0.05$ , 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. 2B; sign test:  $p > 0.05$ ; power analysis:  $\alpha = 0.05$ ,  $n = 22$ ). In the subset of 37 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. 2C; sign test:  $p > 0.05$ ; power analysis:  $\alpha = 0.05$ ,  $n = 12$ ).

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 ALs mated at a higher frequency than those with smaller traits (Fig. 3; GL:  $t_{78} = -3.171$ ,  $p = 0.002$ ; 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 correlated with mating success for gynes (Fig. 3; TW:  $t_{78} = 0.339$ ,  $p = 0.736$ ; TL:  $t_{74} = -1.571$ ,  $p = 0.121$ ; HTL:  $t_{78} = -1.092$ ,  $p = 0.278$ ; CL:  $t_{80} = -0.174$ ,  $p = 0.863$ ; mass:  $t_{82} = 0.765$ ,  $p = 0.446$ ). The number of significant correlations 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 vs. non-nestmate males. The mean length of a mounting for nestmates was 78.37 s, and for non-nestmates 63.13 s; these means are not significantly different (two-tailed  $t$ -test:  $t_{36} = 0.852$ ,  $p = 0.401$ ).

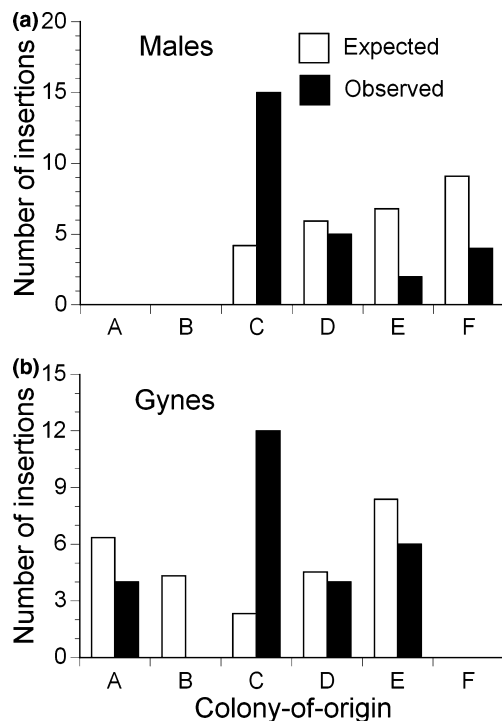
### Colony-of-Origin

We found a strong association between the number of insertions a male obtained and the colony from which it originated. Specifically, males from colony C showed a higher than expected number of matings (Fig. 4A; males:  $\chi^2_3 = 34.2$ ,  $p < 0.001$ ). 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. 4B; gynes:  $\chi^2_4 = 46.6$ ,  $p < 0.001$ ).

### Discussion

The goal of this study was to investigate mating behavior and mating success in the polyandrous social wasp *V. maculifrons*. We wished to test the 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 among 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.



**Fig. 4:** Expected and observed numbers of insertions for *Vespula 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.

### 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 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 the variation in the 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

Although 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). 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 (Wiernasz et al. 1995; Abell et al. 1999; Oberstadt & Heinze 2003). In some taxa, such as the lekking ant

species *P. occidentalis*, male size is a strong indicator of male mating success (Wiernasz et al. 1995). Although 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 for 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). Although trait sizes between males that did mate and those that did not mate were not specifically examined in that study, no difference in size of males who mated multiply and those that mated singly was discovered. Thus, it appears that male size may not be as relevant in determining success in mating activities in social insects as it is in other insect and vertebrate taxa (Andersson 1994; Blaukenhorn 2005).

#### Gyne Trait Size and Mating Success

We found that mating success for gynes was positively correlated with gyne gaster length, antennal length, third tergum length, and total body length (Fig. 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 *L. 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 colony productivity during 1 yr of the study. These findings may indicate that female size is an important factor in female mating success in social insects because of 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. 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, although they did not possess the shortest traits either (Table 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, although total BL 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 mate 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 matrilineages 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.

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