BRIEF COMMUNICATION

THE SIGNIFICANCE OF MULTIPLE MATING IN THE SOCIAL WASP *VESPULA MACULIFRONS*

Michael A. D. Goodisman,[1,2] Jennifer L. Kovacs,[1] and Eric A. Hoffman[1,3]

[1]School of Biology, Georgia Institute of Technology, 310 Ferst Drive, Atlanta, Georgia 30332

[2]E-mail: michael.goodisman@biology.gatech.edu

[3]Department of Biology, University of Central Florida, 4000 Central Florida Blvd., Orlando, Florida 32816

The evolution of the complex societies displayed by social insects depended partly on high relatedness among interacting group members. Therefore, behaviors that depress group relatedness, such as multiple mating by reproductive females (polyandry), are unexpected in social insects. Nevertheless, the queens of several social insect species mate multiply, suggesting that polyandry provides some benefits that counteract the costs. However, few studies have obtained evidence for links between rates of polyandry and fitness in naturally occurring social insect populations. We investigated if polyandry was beneficial in the social wasp *Vespula maculifrons*. We used genetic markers to estimate queen mate number in *V. maculifrons* colonies and assessed colony fitness by counting the number of cells that colonies produced. Our results indicated that queen mate number was directly, strongly, and significantly correlated with the number of queen cells produced by colonies. Because *V. maculifrons* queens are necessarily reared in queen cells, our results demonstrate that high levels of polyandry are associated with colonies capable of producing many new queens. These data are consistent with the explanation that polyandry is adaptive in *V. maculifrons* because it provides a fitness advantage to queens. Our research may provide a rare example of an association between polyandry and fitness in a natural social insect population and help explain why queens in this taxon mate multiply.

KEY WORDS: Hymenoptera, kin selection, polyandry, polymorphic microsatellite marker, relatedness, social insect, Vespidae.

The females of many animal species mate with multiple males in a single reproductive cycle (polyandry). This is surprising because polyandry potentially carries considerable costs to females including lost time, wasted energy output, an increased risk of predation, and a higher probability of contracting disease (Arnqvist and Nilsson 2000; Jennions and Petrie 2000; Simmons 2001; Zeh and Zeh 2001). In highly social animals, such as social insects (i.e., ants, social bees, social wasps, and termites), polyandry has even greater consequences than in nonsocial animals.

The effects of polyandry are particularly far-reaching in social insects because variation in the number of mates per queen lowers the relatedness among queen offspring, potentially decreasing the benefits associated with cooperation and leading to conflict among subfamilies (Boomsma and Ratnieks 1996; Crozier and Pamilo 1996; Frank 1998; Queller and Strassmann 1998). Nevertheless,

queens of several social hymenopteran species regularly mate with multiple males (Strassmann 2001). The occurrence of polyandry in these social insect taxa suggests that multiple mating provides some benefits to queens that offset the potential costs associated with lowered relatedness among nest mates.

Theoretical and empirical research has generated a diversity of hypothetical benefits that may explain the evolution of polyandry in social insects (Boomsma and Ratnieks 1996; Crozier and Fjerdingstad 2001; Strassmann 2001). For example, queens may mate multiply simply to obtain sufficient sperm for reproducing throughout their lifetime (Cole 1983; Fjerdingstad and Boomsma 1998). Alternatively, the genetic variation generated in the queen's female offspring through polyandry may be adaptive. For instance, genetically diverse offspring may be more able to stave off parasites or may have higher fitness as a group under

changeable environmental conditions (Baer and Schmid-Hempel 1999, 2003; Tarpy 2003; Tarpy and Seeley 2006). Polyandry may also reduce conflict over sex ratio between queens and workers within colonies (Moritz 1985; Boomsma and Grafen 1991; Ratnieks and Boomsma 1995; Sundström and Ratnieks 1998) and may decrease conflict related to whether the queen or workers should produce males (Ratnieks 1988). However, despite the plethora of hypotheses proposed to explain the evolution of polyandry, few examples of naturally occurring increases in fitness connected with queen mate number have been uncovered in social insects (for a rare example, see Cole and Wiernasz 1999).

The purpose of this study was to investigate if polyandry was associated with variation in fitness in the social wasp *Vespula maculifrons*. *Vespula maculifrons*, commonly known as the eastern yellowjacket, is found throughout the eastern half of the United States (Akre et al. 1980). A new *V. maculifrons* colony is founded by a single, multiply mated queen in the spring (*V. maculifrons* natural history reviewed by Spradbery 1973; Edwards 1980; MacDonald and Matthews 1981; Greene 1991). The queen constructs the incipient nest and rears the first cohort of workers. Once the workers mature, they take over the task of colony maintenance. However, the queen remains wholly responsible for the production of all offspring within the nest as long as she is present. The workers do not mate and therefore can never produce female offspring. In addition, workers only produce males if the colony becomes queenless (Ross 1985, 1986; Ross and Carpenter 1991; Goodisman et al. 2007).

Workers expand the nest by constructing worker cells throughout the spring and summer (Spradbery 1973; Edwards 1980). At the end of the summer the colony begins to produce males and new reproductive queens (gynes). Males generally emerge before gynes within colonies, because males are usually reared in worker cells, which are already present. In contrast, gynes must be reared in specially constructed, larger queen cells (Fig. 1). *Vespula maculifrons* colonies produce hundreds of gynes and males in a season (MacDonald and Matthews 1981). In addition, colonies typically survive only one year so the number of sexuals produced at the end of the season represents the entire reproductive output of the colony and, by extension, the queen.

We investigated the hypothesis that *V. maculifrons* queens mate multiply because polyandry leads to direct fitness benefits. Specifically, we predicted that if polyandry is adaptive then queens mated to many males should head more successful colonies than queens mated to few males. To test this prediction, we determined if queen mate number was associated with the number of cells produced by colonies, which is directly associated with colony output (Spradbery 1973; MacDonald and Matthews 1981; Greene 1991). Our results indicate that queen mate number may affect fitness in *V. maculifrons*. We discuss this finding in relation to the evolution of polyandry in social taxa.
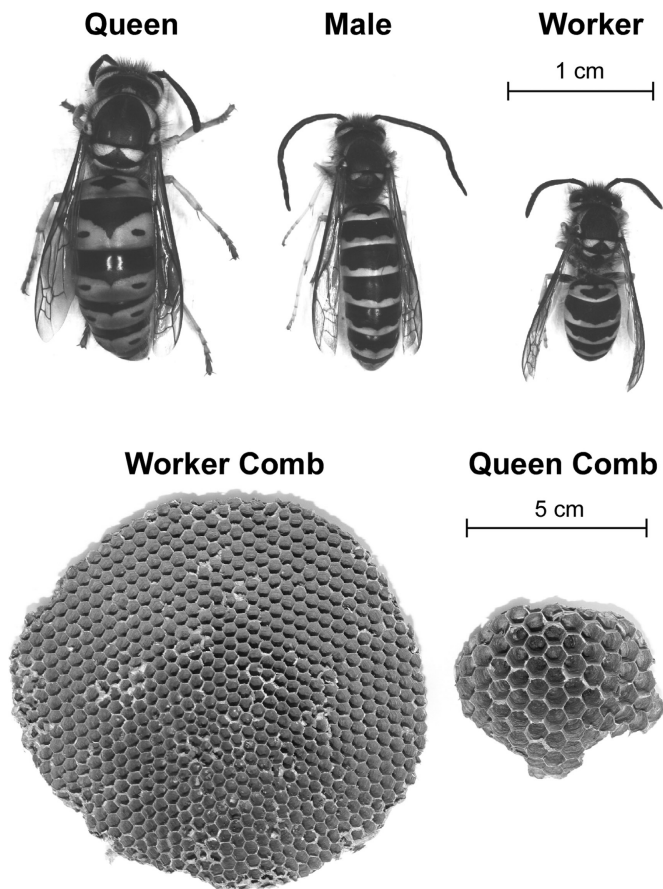

Queen   **Male**   **Worker**

1 cm

Worker Comb   **Queen Comb**

5 cm

Figure 1. *Vespula macuilifrons* **castes and comb. Queens initiate new nests and reproduce within colonies. Males partake in few tasks and exist solely to mate with new queens. Workers maintain the colony and do not reproduce when the queen is present. All workers and the vast majority of males are reared in worker cells. In contrast, new queens are necessarily reared in specially constructed queen cells.**

Methods

We sampled *V. maculifrons* wasps from 28 colonies in and around Atlanta, Georgia, in 2004–2005. Six colonies contained only adult workers, seven colonies contained both adult workers and males, and 15 colonies contained adult workers, males, and gynes (Table 1). We estimated the number of worker and queen cells in each of the 28 nests we collected. This procedure entailed counting the number of cells that made up the width (w) and length (l) of the worker or queen comb and then applying the formula for calculating the area of an ellipse (i.e., $w \times l \times \pi / 4$), which roughly conforms to the shape of a comb in this species. We confirmed that this method gave reasonably accurate results by comparing the number of cells obtained by the estimation technique to the number of cells obtained by direct counting for a subset of combs.

We genotyped 42.79 ± 24.17 (mean $\pm$ SD, Table 1) workers from each colony, as well as the reproductive queen heading

Table 1. Descriptive data from 28 *V. maculifrons* colonies sampled in this study, including the sampling date, the castes of individuals residing in each colony (W=worker, M=male, G=gyne), and the estimated number of worker, queen, and total cells that made up the nest. The number of workers genotyped from each colony (*N*) and the observed (*k*) and effective (k_{e3}) number of males mated to the queen heading the colony are also provided.

Colony ID	Date	Castes	Worker Cells	Queen Cells	Total Cells	N	k	k_{e3}
8	9 Sep 04	W	7532	0	7532	40	5	4.88
13	15 Sep 04	W, M	3530	134	3664	30	4	3.60
15	15 Sep 04	W	3210	0	3210	40	5	3.52
16	24 Sep 04	W	1685	0	1685	33	8	8.82
17	24 Sep 04	W	825	0	825	24	5	3.34
18	28 Sep 04	W	4714	311	5025	32	5	3.17
19	1 Oct 04	W	1544	51	1595	40	6	5.24
23	1 Oct 04	W, M	7511	0	7511	36	3	3.06
24	1 Oct 04	W, M	1256	222	1478	37	6	5.65
25	1 Oct 04	G, W, M	5592	460	6052	39	6	4.79
27	11 Oct 04	W, M	5536	308	5844	40	6	5.70
28	11 Oct 04	W, M	2053	546	2599	39	6	6.45
29	12 Oct 04	G, W, M	3882	595.5	4477.5	39	3	2.61
33	19 Oct 04	W, M	6533	0	6533	38	5	4.48
34	19 Oct 04	G, W, M	5885	731	6616	117	8	7.44
35	19 Oct 04	G, W, M	5856	177	6033	36	5	4.71
36	19 Oct 04	G, W, M	4629	169	4798	18	5	5.92
37	21 Oct 04	W, M	3108	662	3770	33	6	5.19
40	27 Oct 04	G, W, M	1292	21	1313	39	4	3.81
41	27 Oct 04	G, W, M	6491	1187	7678	115	6	5.61
43	27 Oct 04	G, W, M	4794	163	4957	36	6	3.76
44	28 Oct 04	G, W, M	4235	3457	7692	95	5	4.80
45	28 Oct 04	G, W, M	8944	2551	11,495	37	5	4.57
46	5 Nov 04	G, W, M	2139	123	2262	35	5	3.19
53	27 Oct 05	G, W, M	2920	675	3595	37	5	4.45
54	27 Oct 05	G, W, M	5631	2026	7657	37	6	6.06
56	5 Nov 05	G, W, M	8711	1447	10,158	30	7	5.32
57	5 Nov 05	G, W, M	3553	732	4285	26	6	6.52
Mean			4413.96	598.16	5012.13	42.79	5.43	4.88

eight of the colonies, at the seven polymorphic microsatellite loci LIST2003, LIST2004, LIST2013, LIST2019, LIST2020, VMA-3, and VMA-6 (Daly et al. 2002; Hasegawa and Takahashi 2002). The allele frequencies at each locus were estimated by calculating the frequencies within each colony and then taking the mean over all colonies (i.e., weighting colonies equally). The allele frequencies were used to estimate the expected heterozygosities at each locus. We also calculated the nondetection error for this population, which is the probability of two males having the same genotype and, therefore, producing diploid offspring that could not be differentiated if they mated with the same queen (Boomsma and Ratnieks 1996).

The genotypes of the workers within nests were used to reconstruct the genotype of the queen and her male mates from each colony. We also calculated the effective number of mates for each queen (k_{e3}) using the sample size correction proposed by Nielsen et al. (2003). The metric k_{e3} combines information on the number

of times a queen mates and the unequal contributions of a queen's male mates to offspring (Boomsma and Ratnieks 1996). k_{e3} describes the amount of genetic variability of diploid individuals within colonies and will be less than the actual mate number of queens if siring success varies among paternal males.

We tested if the number of cells produced by colonies covaried positively with the number of mates per queen, as would be expected if polyandry provided a benefit to queens and their colonies. Spearman's correlation coefficient was used to assess the relationship between number of cells within a nest and k_{e3}. Statistical calculations were conducted using the software program JMP.

Results

Nests showed a wide variation in cell number and were constructed of 4413.96 ± 2293.42 and 598.16 ± 844.14 (mean $\pm$ SD) worker

and queen cells, respectively (Table 1). The number of worker and queen cells produced by mature colonies (those containing adult sexuals) did not depend on the sampling day ($n = 18$, only nests from 2004 sampling year used; Spearman's correlation coefficient; worker cells, $r_S = 0.0636$, $P = 0.8020$; queen cells, $r_S = 0.2582$, $P = 0.3009$), suggesting that time of year was not a contributing factor to our analyses, because mature colonies were sampled relatively late in the reproductive season. In addition, the number of worker cells and queen cells within nests were not significantly correlated ($n = 18$, $r_S = 0.2271$, $P = 0.3096$).

We found that the seven microsatellite markers used for our study were highly variable. The expected heterozygosities of the loci ranged from 0.722 to 0.937. Consequently, the probability of two males having the same multilocus genotype (nondetection error) was very small ($\ll 0.0001$), indicating that the variability of our markers did not impede our ability to identify male mates of queens (Boomsma and Ratnieks 1996).

As expected, the genotypes of all workers within colonies were consistent with having been produced by a single queen. Moreover, all queens mated multiply. Queen mate number in our sampled nests ranged from three to eight, with a mean of 5.43 ± 1.16 (Table 1). The effective mate number of queens, k_{e3}, was lower than the actual mate number (Table 1; range of k_{e3} was 2.61 to 8.81; mean $\pm$ SD of 4.88 ± 1.42) and not substantially different from prior studies in this species (Ross 1986). Finally, the genotype of the queen when the queen was recovered from the colony was consistent with the genotypes of the resident workers indicating that queen takeover had not occurred in the recent history of these colonies (Matthews 1982; Spradbery 1991).

We investigated if there was a relationship between queen mate number and the number of cells within nests. We first determined if a direct relationship existed between the number of worker cells within nests and queen effective mate number. However, we found no significant association between worker cell number and k_{e3} in all colonies ($n = 28$, $r_S = 0.0323$, $P = 0.8704$) or mature colonies only ($n = 22$, $r_S = 0.0390$, $P = 0.8633$, Fig. 2).

There was a fairly strong but nonsignificant tendency for a positive relationship between k_{e3} and the number of queen cells in all (immature and mature) of the sampled colonies ($n = 28$, $r_S = 0.3525$, $P = 0.0658$). More importantly, we discovered a significant association between the number of queen cells produced and k_{e3} in mature colonies ($n = 22$, $r_S = 0.5021$, $P = 0.0173$, Fig. 2). The relationship is nonlinear (Fig. 2A) and appears to be more consistent with a threshold effect, whereby colonies headed by queens with k_{e3} roughly less than 4.0 produced few queen cells and colonies headed by queens with k_{e3} roughly greater than 4.0 often produced many queen cells. Indeed, of the 16 colonies that produced the highest number of queen cells, 15 were headed by queens effectively mated to more than four males, whereas only one of the six colonies that produced the fewest number of
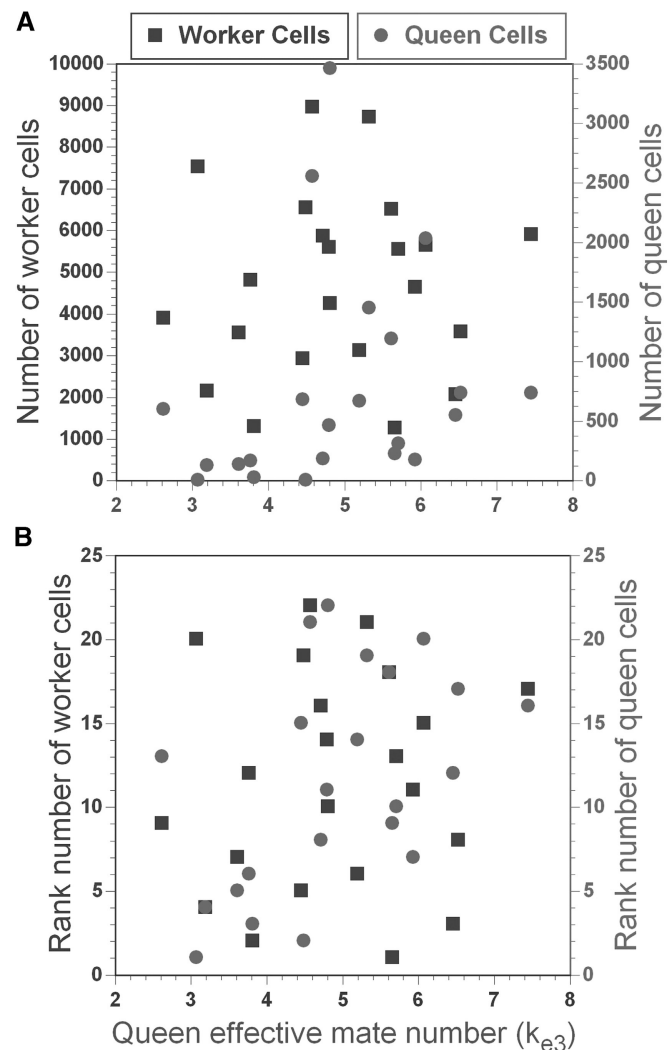

Figure 2. Relationship between (A) absolute number of cells and (B) rank number of cells (ranks range from 1 to 22 with colony of rank 1 containing the fewest cells and colony of rank 22 containing the most cells) produced by mature *V. maculifrons* colonies and the effective mate number of queens heading colonies (k_{e3}). Queens mated to many males headed colonies that produced more queen cells than queens mated to few males.

queen cells was headed by a queen effectively mated more than four times. These proportions differed significantly as judged by Fisher's exact test ($n = 22$, $P = 0.0013$).

We also note that the variance in the number of queen cells increased with effective mate number (Fig. 2). To determine if this relationship was significant, we divided colonies into two groups based on whether the queen effectively mated with four males. Using these criteria we found that the variability of these two classes differed significantly ($k_{e3} < 4.0$, $n = 6$, SD $= 217.10$; $k_{e3} > 4.0$, $n = 16$, SD $= 966.69$; $F_{15,5} = 0.5021$, $P = 0.0038$) and that increased queen mate number was associated with greater variation in queen cell number.

Finally, we investigated if there was a relationship between the timing of reproduction of colonies and queen effective mate number. Specifically, we determined if there was a relationship between whether colonies had queen cells present and k_{e3}. Such a relationship might exist if we sampled colonies early in the reproductive season and colonies headed by queens that mated with few males had not started their reproductive phase. However, we failed to detect significant differences between k_{e3} for colonies that had and had not initiated their reproductive phase ($F_{1,26} = 0.1409, P = 0.7104$). Thus our data do not provide evidence that effective mate number is associated with timing of colony reproductive cycle.

Discussion

The purpose of this study was to determine if polyandry was advantageous for *V. maculifrons* social wasp queens. The key result arising from our investigation is that there was a direct relationship between number of queen cells produced by colonies and effective mate number of queens (k_{e3}). In contrast, there was no significant relationship between number of worker cells produced by colonies and k_{e3}. The relationship between queen cell number and k_{e3} indicates that queens mated to many males headed colonies with greater new-queen (gyne) producing capacity. Moreover, the lack of relationship between worker cell number and k_{e3} suggests that male-producing capacity of colonies was unaffected by queen mate number, because males are reared in worker cells (Spradbery 1973; MacDonald and Matthews 1981).

We also discovered that colonies headed by queens mated to many males showed a higher variance in queen cell production than colonies headed by queens mated to few males. The decreased variability in queen cell number in the colonies headed by queens mated to few males resulted from the fact that such queens almost always headed colonies that produced very few queen cells (Fig. 2A). In contrast, queens mated to many males headed colonies that showed a wide range in queen cell number. The underlying cause of this relationship is unclear. It is possible that some colonies headed by queens mated many times were sampled relatively early in the season and therefore contained relatively few queen cells. Alternatively, some queens mated to many males may face other ecological constraints (e.g., lack of space for building new comb) that may affect the construction of queen cells.

We were somewhat surprised not to detect a significant correlation between the number of worker cells and the number of queen cells produced by colonies. The number of worker cells within colonies is strongly correlated with the number of workers produced by colonies in *V. maculifrons* (MacDonald and Matthews 1981). And we expected that a larger worker population would be capable of producing more queen cells. Moreover, the lack of correlation between number of queen and worker cells stands in contrast to some theories that suggest that production of female sexuals in social insect taxa should be tied to overall productivity of colonies (Nonacs 1986). Thus, it is possible that such a correlation exists in *V. maculifrons* but that we lacked the power to detect it.

However, it is also possible that a direct relationship between number of worker cells and queen cells in this species is weak or nonexistent. Indeed, we are not the first to note that the factors affecting the production of queen cells in *Vespula* colonies are likely to be complex. More than 30 years ago, Spradbery (1973, p. 240) noted that "The construction of queen cells in individual colonies occurs at widely different times during the season and it appears unlikely that their initiation is made in response to seasonal factors such as photoperiod. The size of the colony is probably not critical either because queen cells are found in colonies of widely differing sizes. . .." These observations, as well as our results, suggest that the production of queen cells is not necessarily tightly tied to simple factors such as the number of workers present within the colony. Moreover, the lack of correlation between queen and worker cell number implies that there may not be a trade-off for colonies between producing many gynes and producing many workers and males.

Regardless, if the number of gynes produced by colonies is directly related to the number of queen cells produced by nests, as is the case for the relationship between the number of workers produced by colonies and the number of worker cells (MacDonald and Matthews 1981), then our results suggest that queens mated to many males produce more gynes than queens mated to few males. It would be tempting to further suggest that the number of males produced by colonies does not depend on queen mating frequency, because we uncovered no relationship between number of worker cells, which are typically used to rear males, and k_{e3}. This may be true, but it is difficult to infer the numbers of males produced by colonies. The reason why the relationship between number of worker cells and number of males produced by colonies is unclear is that during the reproductive phase of the colony, worker cells are used to rear both workers and males. In the absence of other information, we may expect all colonies to rear equal proportions of worker and males in their worker cells. However, it is possible that colonies adjust the number of males they produce by rearing different ratios of workers to males. In principle, such biasing might lead to a negative or positive relationship between number of worker cells and the number of males produced by colonies.

Interestingly, the relationship between new-queen producing capacity and k_{e3} uncovered here is not consistent with relatedness-based sex ratio biasing by workers found in other hymenopteran social insects (Queller and Strassmann 1998; Chapuisat and Keller 1999; Mehdiabadi et al. 2003). Such biasing generally results from variation in relatedness of workers to the offspring they rear

and may lead to workers investing relatively highly in females in colonies headed by queens mated to few males in a population in which queens show variation in mate number (Boomsma and Grafen 1991; Ratnieks and Boomsma 1995). This connection between relatedness and sex ratio does not seem able to explain the patterns in *V. maculifrons*. Adaptive sex-ratio biasing predicts that queens mated to many males should produce relatively male-biased sex ratios if workers adaptively bias sexual production in their favor. In contrast, our data suggest that *V. maculifrons* queens mated to many males produce relatively female-biased sex ratios, if we assume that all colonies use their worker cells to rear the same proportion of males. However, a direct examination of the adult sex ratios produced by colonies is needed before final conclusions can be made.

There are several potential causal explanations for the observed relationship between queen mate number and number of queen cells produced by colonies. For example, it is possible that queen mate number is somehow associated with the timing of reproduction in *V. maculifrons*. However, our data do not support this hypothesis. Specifically, we failed to find a difference in the estimates of k_{e3} for queens that headed colonies that either had or had not initiated their reproductive phase. We note, however, that our data were not collected to test this hypothesis. This question might be more accurately addressed by specifically seeking out colonies that had just initiated their reproductive phase (such colonies contain only sexual larvae or pupae, but no sexual adults) and determining if there was a relationship between k_{e3} and date of collection for these colonies.

Another explanation for the relationship is that colonies that produce many queen cells are headed by high-quality queens. Such queens may mate with many males because they would be attractive to breeding males (Bonduriansky 2001). These high-quality queens could also head colonies capable of producing many queen cells. In this case, high mate number would not cause enhanced colony fitness. Rather, both high colony fitness and high mate number would result from some other aspect of queen phenotype (Fjerdingstad and Keller 2004).

Another explanation for the observed relationship between queen cell number and k_{e3} is that the amount of sperm stored by queens mated to different numbers of males varies. Specifically, queens mated to few males may possess insufficient sperm to produce large numbers of diploid gynes (Cole 1983; Stein and Fell 1994; Fjerdingstad and Boomsma 1998). Such queens may signal to workers that the construction of queen cells would be unnecessary. These queens may be relegated to producing haploid males at the end of the season. This hypothesis, although possible, would seem unlikely to explain the observed relationship, because *Vespula* queens would be expected to possess sufficient sperm to produce the 15,000–30,000 (Spradbery 1973) individuals generated during a *Vespula* colony's lifetime.

Alternatively, the association between queen mate number and queen cell production may result from benefits to colonies derived from the high genetic diversity among queen offspring (Crozier and Fjerdingstad 2001). Such increased genetic diversity may lead to increased disease resistance, as has been found in other social insect taxa (Schmid-Hempel 1994; Baer and Schmid-Hempel 1999, 2003; Brown and Schmid-Hempel 2003; Palmer and Oldroyd 2003; Tarpy 2003; Hughes and Boomsma 2004; Tarpy and Seeley 2006). In this case, colonies headed by queens mated to few males may suffer costs of diseases and not be able to produce many queen cells. We did not observe any obvious diseases plaguing our sampled *V. maculifrons* colonies. Moreover, one would expect that worker production would also be affected if colonies suffered from diseases related to a paucity of colony genetic diversity. Nevertheless, it is possible that colonies ailed from diseases that were not easily detectable and affected gyne production alone.

Another explanation for why queens mated to many males headed colonies that produced many queen cells is that such colonies contained a more efficient workforce. Workers produced by queens mated many times may be more efficient as a group, because they would be more genetically variable, and therefore more capable of proficiently completing a diversity of tasks (i.e., genetic polyethism; Crozier and Fjerdingstad 2001; Fjerdingstad et al. 2003). Direct and indirect evidence for genetic polyethism has been uncovered in other social insects (Fraser et al. 2000; Hughes et al. 2003; Jones et al. 2004; Schwander et al. 2005). Thus, it is also possible that workers produced by *V. maculifrons* queens mated to many males are ultimately able to build more queen cells and produce more gynes.

A related hypothesis for the observed relationship is that workers within colonies headed by queens mated to many males specialize on producing gynes, because these gynes will have relatively high fitness. This may be the case if queens mated to many males produce daughters that are genetically more variable than queens mated to few males, and genetically variable daughters better survive as a group (Crozier and Page 1985). That is, colonies headed by queens mated to few males may "choose" not to produce gynes, which would be unsuccessful relative to those produced by colonies headed by queens mated to many males. These former colonies could compensate for their substandard gynes by producing more males, for example, which would be as genetically variable as those produced by colonies headed by queens mated to many males.

Regardless of the potential mechanism underlying the observed association between queen mate number and number of queen cells produced, polyandry may be adaptive in *V. maculifrons* because it leads to increased capacity of colonies to produce new queens. This would represent a rare instance of a fitness benefit associated with higher rates of polyandry in a natural, unmanipulated

social insect population (see Cole and Wiernasz 1999; Wiernasz et al. 2004 for another example). In contrast, studies in *Apis* and *Bombus* have uncovered benefits to polyandry under manipulated conditions (e.g., Baer and Schmid-Hempel 1999, 2003; Fewell and Bertram 2002; Tarpy 2003; Jones et al. 2004; Tarpy and Seeley 2006). Other examples of overt fitness benefits associated with polyandry in social insects occur under unusual circumstances involving hybridization (Julian et al. 2002; Volny and Gordon 2002; Cahan and Keller 2003) or resulting from the mitigating of conflict among colony members (i.e., sex-ratio biasing or worker policing; Ratnieks 1988; Boomsma 1996; Sundström et al. 1996; Sundström and Ratnieks 1998; Chapuisat and Keller 1999; Bourke 2001; Mehdiabadi et al. 2003). However, instances of naturally occurring variation in fitness that are directly associated with rates of polyandry, as we may have uncovered, are relatively rare.

Nevertheless, the veracity of the hypothesis that polyandry leads to increased fitness of *V. maculifrons* queens needs to be investigated further. Specifically, additional years of analysis would help establish if the relationship we observed in 2004 and 2005 is stable and widespread. Moreover, one might expect to find similar trends in other *Vespula* species, which tend to show similar life-history characteristics to *V. maculifrons* (Spradbery 1973; Greene 1991; Foster and Ratnieks 2001). Finally, experimental manipulation of mate number, as have been conducted in social bees (e.g., Baer and Schmid-Hempel 1999, 2003; Tarpy 2003; Tarpy and Seeley 2006), would help elucidate the causal relationship between number of mates and queen reproductive success in *V. maculifrons*.

LITERATURE CITED

Akre, R. D., A. Greene, J. F. MacDonald, P. J. Landolt, and H. G. Davis. 1980. The yellow jackets of America north of Mexico. U. S. Department of Agriculture.

Arnqvist, G., and T. Nilsson. 2000. The evolution of polyandry: multiple mating and female fitness in insects. Anim. Behav. 60:145–164.

Baer, B., and P. Schmid-Hempel. 1999. Experimental variation in polyandry affects parasite loads and fitness in a bumble-bee. Nature 397:151–154.

———. 2003. Bumblebee workers from different sire groups vary in susceptibility to parasite infection. Ecol. Lett. 6:106–110.

Bonduriansky, R. 2001. The evolution of male mate choice in insects: a synthesis of ideas and evidence. Biol. Rev. 76:305–339.

Boomsma, J. J. 1996. Split sex ratios and queen-male conflict over sperm allocation. Proc. R. Soc. Lond. B 263:697–704.

Boomsma, J. J., and A. Grafen. 1991. Colony-level sex-ratio selection in the eusocial Hymenoptera. J. Evol. Biol. 4:383–407.

Boomsma, J. J., and F. L. W. Ratnieks. 1996. Paternity in eusocial Hymenoptera. Phil. Trans. R. Soc. Lond. 351:947–975.

Bourke, A. F. G. 2001. Reproductive skew and split sex ratios in social Hymenoptera. Evolution 55:2131–2136.

Brown, M. J. F., and P. Schmid-Hempel. 2003. The evolution of female multiple mating in social Hymenoptera. Evolution 57:2067–2081.

Cahan, S. H., and L. Keller. 2003. Complex hybrid origin of genetic caste determination in harvester ants. Nature 424:306–309.

Chapuisat, M., and L. Keller. 1999. Testing kin selection with sex allocation data in eusocial Hymenoptera. Heredity 82:473–478.

Cole, B. J. 1983. Multiple mating and the evolution of social behavior in the Hymenoptera. Behav. Ecol. Sociobiol. 12:191–201.

Cole, B. J., and D. C. Wiernasz. 1999. The selective advantage of low relatedness. Science 285:891–893.

Crozier, R. H., and E. J. Fjerdingstad. 2001. Polyandry in social Hymenoptera-disunity in diversity? Annals Zool. Fenn. 38:267–285.

Crozier, R. H., and R. E. Page. 1985. On being the right size: male contributions and multiple mating in social Hymenoptera. Behav. Ecol. Sociobiol. 18:105–115.

Crozier, R. H., and P. Pamilo. 1996. Evolution of social insect colonies: sex allocation and kin selection. Oxford Univ. Press, Oxford.

Daly, D., M. E. Archer, P. C. Watts, M. P. Speed, M. R. Hughes, F. S. Barker, J. Jones, K. Odgaard, and S. J. Kemp. 2002. Polymorphic microsatellite loci for eusocial wasps (Hymenoptera: Vespidae). Mol. Ecol. Notes 2:273–275.

Edwards, R. E. 1980. Social wasps: their biology and control. Rentokil, East Grinstead, U.K.

Fewell, J. H., and S. M. Bertram. 2002. Evidence for genetic variation in worker task performance by African and European honey bees. Behav. Ecol. Sociobiol. 52:318–325.

Fjerdingstad, E. J., and J. J. Boomsma. 1998. Multiple mating increases the sperm stores of *Atta colombica* leafcutter ant queens. Behav. Ecol. Sociobiol. 42:257–261.

Fjerdingstad, E. J., P. J. Gertsch, and L. Keller. 2003. The relationship between multiple mating by queens, within-colony genetic variability and fitness in the ant *Lasius niger*. J. Evol. Biol. 16:844–853.

Fjerdingstad, E. J., and L. Keller. 2004. Relationships between phenotype, mating behavior, and fitness of queens in the ant *Lasius niger*. Evolution 58:5.

Foster, K. R., and F. L. W. Ratnieks. 2001. Paternity, reproduction and conflict in vespine wasps: a model system for testing kin selection predictions. Behav. Ecol. Sociobiol. 50:1–8.

Frank, S. A. 1998. Foundations of social evolution. Princeton Univ. Press, Princeton, NJ.

Fraser, V. S., B. Kaufmann, B. P. Oldroyd, and R. H. Crozier. 2000. Genetic influence on caste in the ant *Camponotus consobrinus*. Behav. Ecol. Sociobiol. 47:188–194.

Goodisman, M. A. D., J. L. Kovacs, and E. A. Hoffman. 2007. Lack of conflict during queen production in the social wasp *Vespula maculifrons*. Mol. Ecol. 16:2589–2595.

Greene, A. 1991. *Dolichovespula* and *Vespula*. Pp. 263–305 *in* K. G. Ross, and R. W. Matthews, eds. The social biology of wasps. Comstock Publishing Associates, Ithaca, New York.

Hasegawa, E., and J.-I. Takahashi. 2002. Microsatellite loci for genetic research in the hornet *Vespa mandarinia* and related species. Mol. Ecol. Notes 2:306–308.

Hughes, W. O. H., and J. J. Boomsma. 2004. Genetic diversity and disease resistance in leaf-cutting ant societies. Evolution 58:1251–1260.

Hughes, W. O. H., S. Sumner, S. Van Borm, and J. J. Boomsma. 2003. Worker caste polymorphism has a genetic basis in *Acromyrmex* leaf-cutting ants. Proc. Natl. Acad. Sci. USA 100:9394–9397.

Jennions, M. D., and M. Petrie. 2000. Why do females mate multiply? A review of the genetic benefits. Biol. Rev. 75:21–64.

Jones, J. C., M. R. Myerscough, S. Graham, and B. P. Oldroyd. 2004. Honey bee nest thermoregulation: diversity promotes stability. Science 305:402–404.

Julian, G. E., J. H. Fewell, J. Gadau, R. A. Johnson, and D. Larrabee. 2002. Genetic determination of the queen caste in an ant hybrid zone. Proc. Natl. Acad. Sci. USA 99:8157–8160.

MacDonald, J. F., and R. W. Matthews. 1981. Nesting biology of the Eastern yellowjacket, *Vespula maculifrons* (Hymenoptera: Vespidae). J. Kansas Entomol. Soc. 54:433–457.

Matthews, R. W. 1982. Social parasitism in yellowjackets. Pp. 193–202 *in* P. Jaisson, ed. Social insects in the tropics. Univ. Paris-Nord Paris, France.

Mehdiabadi, N. J., H. K. Reeve, and U. G. Mueller. 2003. Queens versus workers: sex-ratio conflict in eusocial Hymenoptera. Trends Ecol. Evol. 18:88–93.

Moritz, R. F. A. 1985. The effects of multiple mating on the worker-queen conflict in *Apis mellifera* L. Behav. Ecol. Sociobiol. 16:375–377.

Nielsen, R., D. R. Tarpy, and K. Reeve. 2003. Estimating effective paternity number in social insects and the effective number of alleles in a population. Mol. Ecol. 12:3157–3164.

Nonacs, P. 1986. Ant reproductive strategies and sex allocation theory. Q. Rev. Biol. 61:1–21.

Palmer, K. A., and B. P. Oldroyd. 2003. Evidence for intra-colonial genetic variance in resistance to American foulbrood of honey bees (*Apis mellifera*): further support for the parasite/pathogen hypothesis for the evolution of polyandry. Naturwissenschaften 90:265–268.

Queller, D. C., and J. E. Strassmann. 1998. Kin selection and social insects. Bioscience. March: 165–175.

Ratnieks, F. L. 1988. Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. Am. Nat. 132:217–236.

Ratnieks, F. L. W., and J. J. Boomsma. 1995. Facultative sex allocation by workers and the evolution of polyandry by queens in social Hymenoptera. Am. Nat. 145:969–993.

Ross, K. G. 1985. Aspects of worker reproduction in four social wasp species (Insecta: Hymenoptera: Vespidae). J. Zool., London A 205:411–424.

———. 1986. Kin selection and the problem of sperm utilization in social insects. Nature 323:798–800.

Ross, K. G., and J. M. Carpenter. 1991. Population genetic structure, relatedness, and breeding systems. Pp. 451–479 *in* K. G. Ross, and R. W. Matthews, eds. The social biology of wasps. Comstock Publishing Associates, Ithaca, New York.

Schmid-Hempel, P. 1994. Infection and colony variability in social insects. Philos. Trans. R. Soc. Lond. 346:313–321.

Schwander, T., H. Rosset, and M. Chapuisat. 2005. Division of labour and worker size polymorphism in ant colonies: the impact of social and genetic factors. Behav. Ecol. Sociobiol. 59:215–221.

Simmons, L. W. 2001. Sperm competition and its evolutionary consequences in the insects. Princeton Univ. Press, Princeton, NJ.

Spradbery, J. P. 1973. Wasps: an account of the biology and natural history of solitary and social wasps. Sidgwick & Jackson, London.

———. 1991. Evolution of queen number and queen control. Pp. 336–388 *in* K. G. Ross, and R. W. Matthews, eds. The social biology of wasps. Comstock Publishing Associates, Ithaca, New York.

Stein, K. J., and R. D. Fell. 1994. Correlation of queen sperm content with colony size in yellowjackets (Hymenoptera: Vespidae). Environ. Entomol. 23:1497–1500.

Strassmann, J. 2001. The rarity of multiple mating by females in the social Hymenoptera. Insect. Soc. 48:1–13.

Sundström, L., M. Chapuisat, and L. Keller. 1996. Conditional manipulation of sex ratios by ant workers: a test of kin selection theory. Science 274:993–995.

Sundström, L., and F. L. W. Ratnieks. 1998. Sex ratio conflicts, mating frequency, and queen fitness in the ant *Formica truncorum*. Behav. Ecol. 9:116–121.

Tarpy, D. R. 2003. Genetic diversity within honeybee colonies prevents severe infections and promotes colony growth. Proc. R. Soc. Lond. B 270:99–103.

Tarpy, D. R., and T. D. Seeley. 2006. Lower disease infections in honeybee (*Apis mellifera*) colonies headed by polyandrous vs monandrous queens. Naturwissenschaften 93:195–199.

Volny, V. P., and D. M. Gordon. 2002. Genetic basis for queen-worker dimorphism in a social insect. Proc. Natl. Acad. Sci. USA 99:6108–6111.

Wiernasz, D. C., C. L. Perroni, and B. J. Cole. 2004. Polyandry and fitness in the western harvester ant, *Pogonomyrmex occidentalis*. Mol. Ecol. 13:1601–1606.

Zeh, J. A., and D. W. Zeh. 2001. Reproductive mode and the genetic benefits of polyandry. Anim. Behav. 61:1051–1063.

Associate Editor: J. Shykoff