


## ORIGINAL ARTICLE

## Novel insights into paternity skew in a polyandrous social wasp

Sarah E. Orr, Nicole A. Hedrick, Kayla A. Murray, Abhinav K. Pasupuleti and Michael A. D. Goodisman 

Georgia Institute of Technology, School of Biological Sciences, Atlanta, Georgia, USA

**Abstract** Females of many species are polyandrous. However, polyandry can give rise to conflict among individuals within families. We examined the level of polyandry and paternity skew in the common eastern yellowjacket wasp, *Vespula maculifrons*, in order to gain a greater understanding of conflict in social insects. We collected 10 colonies of *V. maculifrons* and genotyped workers and prereproductive queens at highly variable microsatellite markers to assign each to a patriline. Genotypic data revealed evidence of significant paternity skew among patrilines. In addition, we found that patrilines contributed differentially to caste production (worker vs. queen), suggesting an important role for reproductive conflict not previously discovered. We also investigated if patterns of paternity skew and mate number varied over time. However, we found no evidence of changes in levels of polyandry when compared to historical data dating back almost 40 years. Finally, we measured a suite of morphological traits in individuals from the most common and least common patrilines in each colony to test if males that showed highly skewed reproductive success also produced offspring that differed in phenotype. Our data revealed weak correlation between paternity skew and morphological phenotype of offspring sired by different males, suggesting no evidence of evolutionary tradeoffs at the level investigated. Overall, this study is the first to report significant paternity and caste-associated skew in *V. maculifrons*, and to investigate the phenotypic consequences of skew in a social wasp. Our results suggest that polyandry can have important consequences on the genetic and social structure of insect societies.

**Key words** castes; eusocial insects; morphology; paternity; polyandry; reproductive conflict

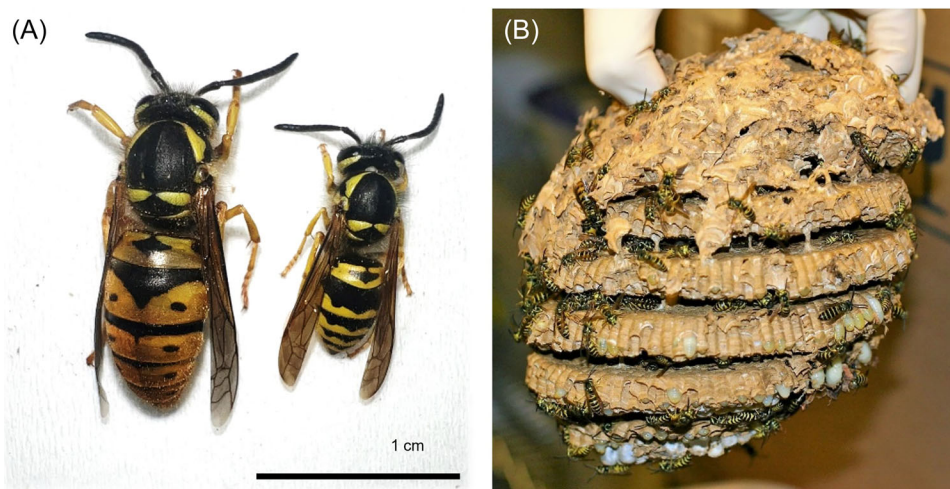
## Introduction

Many insects are polyandrous (Shuker & Simmons, 2014; Snook, 2014; Taylor *et al.*, 2014; Boulton *et al.*, 2018). That is, females mate with multiple males in a single reproductive cycle (Boulton & Shuker, 2013). The evolution of polyandry is surprising because multiple mating has considerable costs to females, such as a loss of

time and energy expended in mating, as well as an increased risk of predation and disease (Boomsma, 2013; Pizzari & Wedell, 2013; Snook, 2014). Thus, substantial research has focused on understanding why some insects have evolved polyandrous mating systems (Baer, 2014; Hare & Simmons, 2019; Fromonteil *et al.*, 2023).

The origin and maintenance of polyandry in highly social insects (i.e., ants, social bees, social wasps, and termites) is of particular interest (Boomsma, 2013; Jaffé, 2014; Baer, 2016). Polyandry has potentially far-reaching evolutionary consequences in insect societies, because multiple mating by females alters the genetic relationships among colony members, thereby changing the

Correspondence: Michael A. D. Goodisman, Georgia Institute of Technology, School of Biological Sciences, 310 Ferst Drive, Atlanta, GA 30332, USA. Fax: 404 385 6311. Email: michael.goodisman@biology.gatech.edu



**Fig. 1** (A) *Vespula maculifrons* gynes (left) and worker (right) wasps. (B) A small, mature *V. maculifrons* nest.

costs of cooperation and conflict (Boomsma, 2013; Jaffé, 2014). Changes in colony genetic diversity arising from multiple mating may ultimately affect decisions by individuals to cooperate, disperse, and reproduce, thereby affecting the evolution of sociality overall (Seeley & Tarpay, 2007; Boomsma, 2013; Baer, 2016; Dillard & Westneat, 2016; Davies & Gardner, 2018; Saga *et al.*, 2020). Notably, however, polyandry is relatively common among social insects and found in approximately one third of social insect species (Baer, 2016). Thus, polyandrous social insects are key taxa to study reproductive conflict arising from polyandrous mating systems (Boomsma, 2013; Fromonteil *et al.*, 2023).

The rearing of new queens (hereafter, gynes) is a critical transition for social insect colonies but may also be an important source of conflict among subfamilies. Workers within colonies headed by polyandrous queens may “encourage” closely related, full siblings to develop into gynes or even intentionally harm unrelated brood. This nepotism is an example of reproductive competition that could be selected for in colonies comprised of multiple subfamilies (Baer, 2016). For example, worker ants in polygynous *Formica fusca* colonies can detect and selfishly favor the rearing of their close kin (Hannonen & Sundström, 2003). Reproductive competition could also occur if workers preferentially care for brood from certain subfamilies. Moreover, sperm competition may occur among the queen’s male mates within a queen’s reproductive organs leading to variation in male reproductive success (Jaffé, 2014; Baer, 2016; Simmons & Wedell, 2020; Degueldre & Aron, 2023). For example, some male ejaculate contains seminal fluid to kill the sperm of other

males present in the queen sexual tract (den Boer *et al.*, 2010).

Here, we investigated patterns of reproductive competition and paternity skew in natural colonies of the polyandrous social wasp, *Vespula maculifrons* (Fig. 1). Vespid wasps show broad variation in social structure and behavior (Hunt & Toth, 2017; Taylor *et al.*, 2018). The social biology and caste system of *Vespula* has also been well-studied (Foster & Ratnieks, 2001), making *Vespula* an ideal taxon to study reproductive competition and paternity skew. Typically, *Vespula* colonies are headed by a single, polyandrous queen (Spradbery, 1973; Ross & Matthews, 1991). The genetic structure of such colonies allows one to study the effects of polyandry in isolation (Holzer *et al.*, 2006). *Vespula* colonies also produce hundreds of new gynes annually, amplifying sample size and statistical power for the study of reproductive output of each colony (Spradbery, 1973; Dyson *et al.*, 2021). And, notably, the genetic structure of *V. maculifrons* colonies have been studied in this geographic area for almost 4 decades (Ross, 1986; Goodisman *et al.*, 2007a, 2007b; Kovacs *et al.*, 2010; Dyson *et al.*, 2021). This historical dataset is a valuable tool to answer questions about long-term changes in the reproductive biology of this species, specifically through the lens of environmental change. Such evolutionary or plastic changes could provide important signals of changes in selective pressures.

This investigation sought evidence for reproductive competition and paternity skew in *V. maculifrons*. We were interested in determining if the different male mates of individual queens contributed differentially to

offspring production. Significant skew would indicate that some males were “winning”, and some “losing”, the competition for reproduction (Nonacs, 2000; Nonacs & Hager, 2011; Jaffé *et al.*, 2012). Therefore, skew among males would represent an important contributor to fitness differences among males. Further, we examined the proportion of queen and worker offspring production by male mates of the queen. Such variation would be expected if reproductive competition occurred between males over caste production (Jaffé *et al.*, 2012). Males should compete to produce gynes rather than worker, because workers are typically sterile (although *V. maculifrons* workers can produce male offspring in some circumstances). Thus, a male that sires more gynes than workers would experience greater fitness than other competing males.

We were also interested in the possible effects of polyandrous mating systems on social phenotypes. Tradeoff theories predict that “cheating” genotypes are held at low frequency within natural populations due to countervailing selective processes (Van Dyken *et al.*, 2011). This “kin selection-mutation balance” hypothesis proposes fitness tradeoffs in male traits but has rarely been tested empirically. In the present study, we tested predictions inspired from this hypothesis by examining if males that were successful in reproductive competition produced progeny with inferior phenotypes. In this case, we tested if males that sired a higher proportion of offspring produced gynes of smaller size, as robust size is associated with gyne viability and reproductive success (Kingsolver & Pfennig, 2004; Kovacs & Goodisman, 2012; Wright *et al.*, 2019). In other words, we explored if males that managed to “cheat” in reproductive competition would suffer some counterbalancing costs in other fitness-related aspects of their genotype.

This investigation thus had 4 specific aims. First, (1) we determined if males showed differential partitioning in reproduction of daughters (i.e., skew). Next, (2) we determined whether the male mates of queens contributed differentially to the worker and gyne castes within colonies (i.e., caste-biased paternity skew). (3) We then examined historical variation in paternity skew in this species with 4 decades of published data. And finally, (4) we tested predictions inspired by “kin selection-mutation balance” theory by examining the phenotypes of offspring produced by males showing different levels of paternity skew. Overall, this study advances our understanding of the nature and consequences of polyandry in highly social insect taxa.

## Materials and methods

### Wasp collection

Ten colonies of *V. maculifrons* wasps were collected in November 2021 and 2022 around Atlanta, Georgia, USA. Briefly, ~100 mL of ether was poured into the colony’s entrance hole to anesthetize the resident wasps. The nests were removed gently using hand trowels. Colonies were placed in a ventilated plastic container and transported to the laboratory. Wasps were euthanized via rapid cooling and stored at  $-20^{\circ}\text{C}$ . Worker and gyne wasps were sorted into sterile microcentrifuge tubes for additional analysis.

### Genetic analysis

We genotyped  $48.00 \pm 3.13$  gynes and  $98.00 \pm 3.65$  (mean  $\pm$  SD) workers from each colony. Individual wasps were weighed, and a single posterior leg was removed and put into a separate sterile tube. We extracted DNA from the leg of each wasp using the Chelex method (Walsh *et al.*, 1991). Samples were dipped into liquid nitrogen and then ground with a sterilized pestle. We then added 250  $\mu\text{L}$  of 5% Chelex solution, vortexed, and heated each sample to  $95^{\circ}\text{C}$  for 30 min. DNA samples were stored at  $-20^{\circ}\text{C}$  and centrifuged before use.

We amplified 6 highly variable microsatellites based on previous studies via PCR in duplex reactions (Table S1) (Foster *et al.*, 2001; Daly *et al.*, 2002; Hasegawa & Takahashi, 2002; Dyson *et al.*, 2022). Using the expected heterozygosity of each locus, we calculated a non-detection error of 0.000249. The nondetection error is the probability that 2 male mates of a queen would have the same multilocus genotype and, therefore, be undetectable as separate males genetically. Therefore, we were able to assign each wasp to a patriline with high confidence. PCR amplification was performed using an Eppendorf Mastercycler and confirmed via visualization on a 3% ethidium bromide agarose gel.

The PCR products from each individual were combined in equal ratios with ROX-labeled ladder and formamide. Each sample was denatured by heating to  $96^{\circ}\text{C}$  for 3 min and cooled on ice briefly. Samples were mailed overnight for fragment analysis at Eton Biosciences. We then identified allele sizes for each locus using the ThermoFisher Connect™ Microsatellite Analysis software. Finally, we used allelic size patterns to manually record patriline for each wasp.

**Table 1** Information on the 10 *Vespa maculifrons* colonies used in this study including date collected and phenotypic sample sizes for workers ( $n^W$ ) and gynes ( $n^G$ ) from rare or common patriline.

Colony	Date collected	$n^W$		$n^G$	
		rare	rare	common	common
1	November 11, 2021	10	2	22	11
2	November 19, 2021	9	5	23	11
3	November 19, 2021	9	7	27	7
4	November 10, 2022	13	4	20	12
5	November 14, 2022	3	7	26	8
6	November 10, 2022	10	2	19	8
7	November 10, 2022	13	8	24	14
8	November 14, 2022	8	7	22	9
9	November 14, 2022	12	7	25	15
10	November 7, 2022	10	9	28	13

### Morphological measurements

We investigated phenotypic differences between individuals produced by “high-skew” males and “low-skew” males (sample size information in Table 1). That is, we measured traits of gyne and worker wasps from the most common and one of the least common patriline identified from each colony. For some colonies, we did not use the least common patriline to ensure a reasonable sample size. We measured 8 morphological traits of each worker and gyne (body mass, body length, forewing length, resilin joint area length, submarginal cell 1 length, femur length, tibia length, and tarsus length) (Ruttner *et al.*, 1978; Radloff *et al.*, 2002; Radloff *et al.*, 2005; Alattal *et al.*, 2014) using an Olympus SZX16 microscope and Teledyne Lumenera Infinity5 software. All wasps within a colony were measured by the same researcher to minimize bias.

### Data analysis

All statistical analyses were performed in JMP Pro 16 software. Genotypic data were analyzed to estimate queen mate number and effective mate number ( $k_e$ ) (Boomsma & Ratnieks, 1997; Nielsen *et al.*, 2003; Goodisman *et al.*, 2007b). We performed a paired *t*-test between the  $k_e$  metric for workers and gynes in order to test if the effective mate number in workers differed from that in gynes. Next, we calculated the paternity skew  $B$  index with 95% confidence intervals using the Skew Calculator (Nonacs, 2000; Nonacs & Hager, 2011). The male contributions to worker: gyne production ratio were analyzed using a Pearson's Chi-Square test to test for “caste-biased paternity skew”. All colonies were analyzed individually and then *P*-

values were combined using Stouffer's combined *P*-value method.

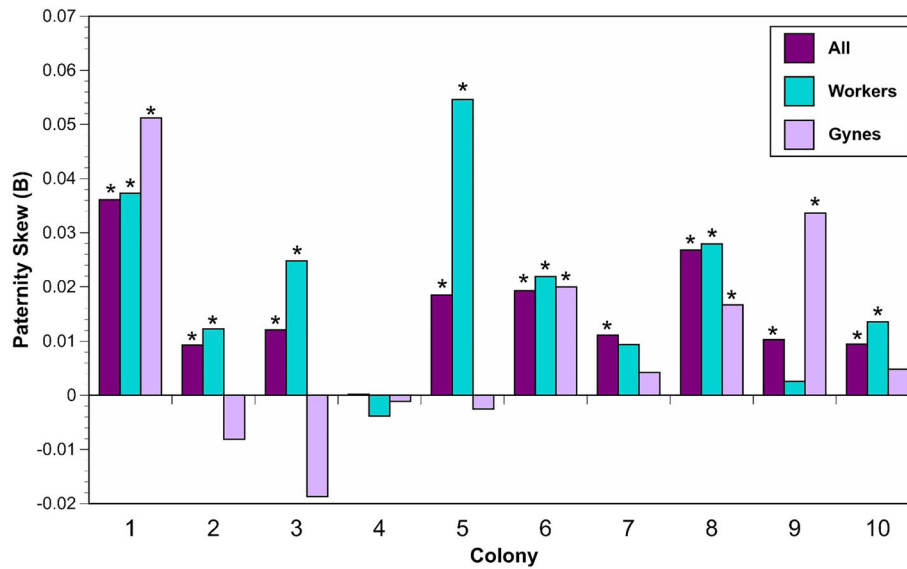
We compared our findings of paternity skew to previous work in *V. maculifrons*. We examined historical data from 1984, 2004, 2006, and 2017 (Ross, 1986; Goodisman *et al.*, 2007a; Kovacs *et al.*, 2008; Dyson *et al.*, 2021). First, we verified that the data conformed to a normal distribution. Then, we tested for differences in mate number, effective mate number, and skew index among sampling years by fitting a model and assigning year as a random effect. Finally, we examined phenotypic differences between common and rare patriline using a statistical model for each morphological trait and caste, using colony as a block to account for environmental variation.

## Results

### Paternity skew

We found that *V. maculifrons* queens mated with 5–8 males (Table 2). Thus, queens were always moderately polyandrous. The overall effective mate number ( $k_e$ ) ranged from 4.77 to 5.19, and was always lower than the actual mate number, indicating that there was some variance in reproductive success of males.

We further investigated if males contributed unequally to daughter production. We found that paternity skew, as measured by the skew index ( $B$ ), was small, but often significant. The male mates of 9 out of the 10 colonies displayed significant paternity skew when considering all offspring together (Fig. 2). Males showed significant paternity skew in gynes in 4 out of 10 colonies (colonies 1, 6, 8, and 9), and significant paternity skew in workers in 7 out of 10 colonies (colonies 1, 2, 3, 5, 6, 8, and 10).



**Fig. 2** Paternity skew ( $B$ ) in workers, gynes, and all individuals combined from 10 *Vespa maculifrons* colonies. \* indicates that 95% confidence intervals do not overlap 0,  $P < 0.05$ .

**Table 2** Queen mate number ( $k$ ) and effective mate number ( $k_e$ ) for workers (W), gynes (G), and the total number of individuals combined (T) for all 10 colonies sampled.

Colony	$k^W$	$k^G$	$k^T$	$k_e^W$	$k_e^G$	$k_e^T$
1	8	7	8	6.14	5.10	5.98
2	6	6	6	5.58	6.29	5.68
3	6	6	6	5.21	6.73	5.59
4	6	6	6	6.13	6.01	5.99
5	7	7	7	5.05	7.09	6.19
6	7	7	7	6.06	6.10	6.16
7	6	6	6	5.67	5.83	5.63
8	8	8	8	6.52	7.01	6.58
9	5	5	5	4.93	4.26	4.75
10	5	5	5	4.68	4.86	4.77
Mean	6.40	6.30	6.40	5.59	5.93	5.73
SEM	0.34	0.30	0.34	0.19	0.29	0.19

Further, we found no evidence of a significant correlation for paternity skew estimates ( $B$ ) in the 2 castes ( $r = 0.093$ ,  $P = 0.80$ ). That is, colonies that showed high skew in gynes did not necessarily show high skew in workers.

*Caste-biased paternity skew*

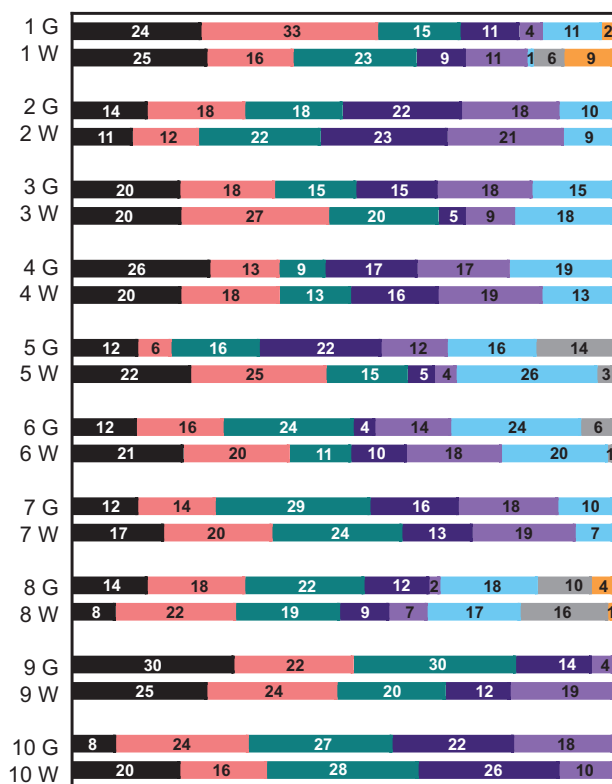
We next investigated if males showed differential success in their contribution to workers versus gynes. Such “caste-biased paternity skew” would indicate that males contributed differentially to worker and gyne production.

We found that some patriline contributed unequally to worker versus gyne production (Figs. 2 and 3). That is, in some cases males produced a significantly higher (or lower) proportion of gynes than workers. The chi-square test  $P$ -value ( $P$ ) for colonies 1–10 was 0.02, 0.89, 0.26, 0.76, 0.0001, 0.12, 0.85, 0.56, 0.12, and 0.25, respectively. Two out of the 10 colonies examined had patriline with significantly different caste production ratios (colony 1,  $P = 0.02$  and colony 5,  $P = 0.0001$ ). Moreover, all colonies showed trends in the direction of differential contribution to workers and gynes. And when we combined information across all colonies, we found highly significant evidence of caste-biased paternity skew ( $P < 0.0001$ ).

We also calculated caste correlations ( $r$ ), which represent the linear correlation between gyne and worker allocation among patriline. That is, we tested if males that sired many workers, also sired many gynes and vice versa. Caste correlations were 0.60, 0.78, 0.49, 0.74, -0.51, 0.21, 0.81, 0.74, 0.92, and 0.26 for colonies 1–10, respectively. In addition, we tested if there were significant differences in the measure of effective mate numbers ( $k_e$ ) in the gyne and worker castes. However, we did not find a statistically significant difference between the metrics in the 2 castes ( $t = 1.13$ ,  $P = 0.28$ ) (Table 2).

*Patterns of paternity skew over time*

We compared the paternity skew ( $B$ ) identified in this study with that detected in previous studies in 2004, 2006,



**Fig. 3** Caste-biased paternity skew in 10 *Vespa maculifrons* colonies; each color represents a different patriline within each colony and numbers represent percentages of each patriline's contribution to gynes (G) or workers (W). Combined  $P < 0.0001$  for all colonies together indicates that *V. maculifrons* shows significant caste-biased paternity skew overall.

and 2017. We found that the magnitude of  $B$  was similar across years (Fig. 4A). Further, we found no differences in queen mate number ( $P = 0.83$ ,  $df = 54$ ) among sampling years reaching back to 1984 (Fig. 4B). Similarly, we observed no significant differences in effective mate number ( $P = 0.44$ ,  $df = 54$ ) among colonies collected in 1984, 2004, 2006, 2017, or 2022 (Fig. 4B).

#### Paternity skew and evolutionary tradeoffs

We hypothesized that males that sired a large proportion of gynes would produce gynes with inferior phenotypes (Van Dyken *et al.*, 2011). Thus, we investigated whether males that showed high (low) overall paternity skew tended to produce relatively small (large) gyne offspring, as predicted if there were trade-off in fitness components for males. We found only 2 traits, forewing length and resilin joint area length, to be significantly different between patrilines in the gyne caste ( $P = 0.033$

and  $P = 0.0087$ , respectively) (Fig. 5 and Table S2). That is, males that produced fewer daughters, produced gynes with longer forewings and longer resilin joint areas than males that sired the most daughters. However, overall, we found little evidence that paternity skew was significantly correlated with offspring phenotype.

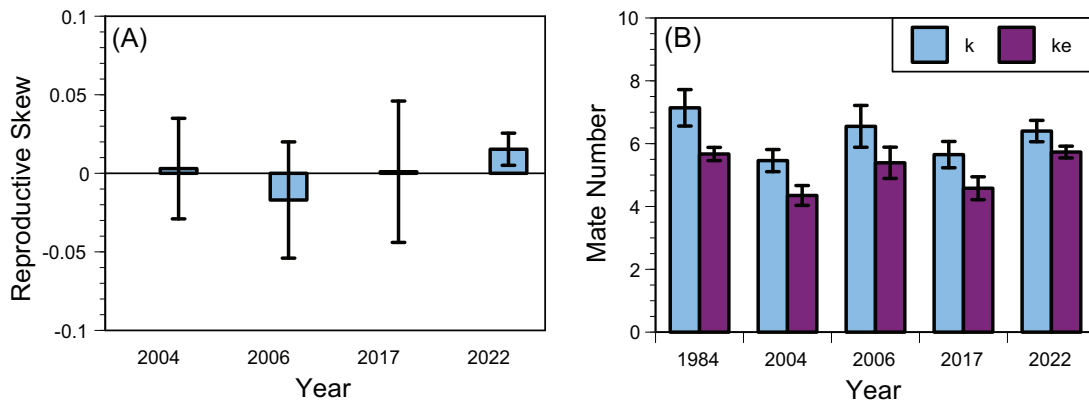
## Discussion

Many social insect queens mate with multiple males. Multiple mating by queens can have important consequences on the fitness of colony members. For example, honeybee queens with higher levels of polyandry tend to produce larger, healthier colonies that are more resistant to disease (Mattila & Seeley, 2007; Seeley & Tarpay, 2007). Moreover, polyandry can affect the function of social insect colonies because polyandry can lead to conflict and reproductive competition. Further, males also may compete for reproductive success either physically, through mating access to gynes, or chemically, by using seminal fluid in sperm competition. Our study used the polyandrous social wasp *V. maculifrons* to investigate important questions about paternity skew and reproductive competition in social insects. We find significant evidence of reproductive competition indicating that polyandry is consequential in this species.

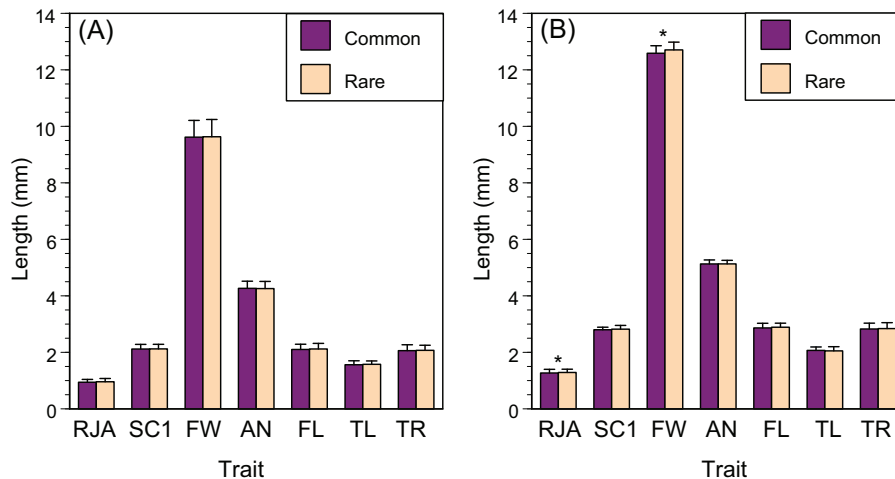
#### Paternity skew

Many polyandrous hymenopteran social insects display skew among male mates (Jaffé *et al.*, 2012). For example, honeybee queens mate many times which results in subsequent paternity skew among workers (Tarpay & Nielsen, 2002; Schlüns *et al.*, 2005; Tarpay *et al.*, 2015; Forfert *et al.*, 2017). Our first goal was to determine if males mated to polyandrous *V. maculifrons* queens showed evidence for paternity skew. We asked if some male mates of queens produced more offspring, regardless of caste, than others. *V. maculifrons* colonies are headed by a single polyandrous queen; thus, it is relatively straightforward to examine the contributions of different male mates to offspring production by genotyping workers and gynes from individual colonies.

We found modest, but statistically significant, levels of paternity skew in most colonies (Fig. 2). Offspring from all but 1 of the 10 colonies examined showed significant skew when information from both gynes and workers were combined. Estimates for skew for gynes and workers individually were also frequently statistically significant, although less often so, presumably due to the lower sample sizes for these individual tests. Notably, the



**Fig. 4** (A) Historical measures of reproductive skew in *Vespula maculifrons*. Statistically significant male skew is evident in 2022 but absent from previous years (Paternity skew  $\pm$  SD) despite similar magnitudes. (B) Distribution of mate number ( $k$ ) and effective mate number ( $k_e$ ) for *V. maculifrons* queens in 5 sampling years. Queen mate number and effective mate number did not differ significantly across years ( $P = 0.83$  and  $P = 0.44$ , respectively).



**Fig. 5** Length of morphological traits in (A) workers and (B) gynes from common and rare patriline. Traits are abbreviated as: resilin joint area length (RJA), submarginal cell 1 length (SC1), forewing length (FW), antenna length (AN), femur length (FL), tibia length (TL), and tarsus length (TR). The majority of traits show no evidence for patriline effects. Error bars represent the standard deviation of the mean. \* indicates significant difference between common and rare patrilines of traits ( $P < 0.05$ ).

actual magnitude of skew, as measured by the metric  $B$ , was quite low. Thus, the paternity skew, though significant, is not particularly strong.

Other *Vespula* wasp species have also been shown to display modest male paternity skew. For example, significant paternity skew was reported in *V. rufa* (Wenseleers *et al.*, 2005) and *V. squamosa* (Hammond & Keller, 2004; Hoffman *et al.*, 2008). However, no evidence for paternity skew was found in *V. germanica* (Goodisman *et al.*, 2002; Hammond & Keller, 2004; Bonckaert *et al.*, 2008) or *V. vulgaris* (Foster & Ratnieks, 2001; Wense-

leers *et al.*, 2005). Interestingly, Vespine wasp species with large colony sizes have evolved relatively low paternity skew (Loope *et al.*, 2014). This suggests that females may control sperm use to increase colony genetic diversity (Jaffe *et al.*, 2012). Notably, our study is the first to report significant paternity skew in *V. maculifrons*. One difference between the current study and past studies in this species is sample size (Dyson *et al.*, 2021). Here, we genotyped up to 150 individuals from each colony, which dramatically increases statistical power to detect paternity skew.

The finding of significant skew in this species raises the question of what mechanisms may lead to differential reproductive success of males. It is possible that there is some level of male precedence in this species. That is, males that mate first, or last, with a gyne may gain some reproductive advantage. Indeed, *Formica truncorum* males engage in “partial sperm clumping” to manipulate number of offspring sired (Sundström & Boomsma, 2000). Similarly, paternity skew controlled temporally has been identified in other species of polyandrous ants (Wiernasz & Cole, 2003; Wiernasz & Cole, 2010). Indeed, temporal variation may help explain paternity skew in *V. maculifrons* since the onset of gyne production occurs much later in the cycle of annual colonies (Johnson *et al.*, 2009).

Males in some social insect species have evolved traits to enhance mating success, such as increased ejaculate size or larger genital morphology (Simmons & García-González, 2008; Simmons & Garcia-Gonzalez, 2011), which could lead to greater reproductive success. Additionally, male wasps may utilize various strategies to produce more offspring. For example, male ejaculate in some ants and bees may contain seminal fluid to eliminate the sperm of competing males in the queen’s reproductive organs (den Boer *et al.*, 2010). Male insects also may compete physically with other males to gain the opportunity to mate with a female. Further, there may be male characteristics that may alter their reproductive success. Larger males may produce more sperm, which could lead to an increased number of offspring. Additionally, males that have been exposed to particularly favorable environments with a highly nutritious diet may have higher quality sperm capable of producing more offspring.

Females may also engage in processes to use the sperm of certain males at higher frequencies. This phenomenon, known as cryptic female choice (Eberhard, 1996), may occur in social insect taxa (Baer, 2015). For example, honeybee and bumblebee queens are capable of actively closing their sting chambers or bursa copulatrix to prevent sperm from entering the spermatheca (Baer *et al.*, 2003; Baer & Schmid-Hempel, 2005). Similarly, some social insects can neutralize seminal fluid of rival males (den Boer *et al.*, 2010) or discard sperm of specific males (Baer, 2011). Interestingly, some queen ants will physically injure or kill males during copula (Allard *et al.*, 2007). All these mechanisms would be expected to be subject to strong levels of selection. But the mechanisms associated with paternity skew in *V. maculifrons*, and most insect species, remains unclear.

### Caste-biased paternity skew

We investigated if male mates of individual *V. maculifrons* queens contributed differentially to gyne versus worker production. That is, we were interested in understanding if males that sired fewer (more) workers also sired fewer (more) gynes, or whether there might be some difference in offspring production for males in the siring of workers and gynes. This question is of particular interest in social insect colonies because males should be under strong selection to sire reproductive gynes rather than mostly sterile workers. So, males should compete to sire gynes, and queens should select particularly “high fitness” males to sire their gyne offspring.

We found that males displayed significant caste-biased skew in some colonies. That is, males displayed significant variation in gyne and worker production within colonies (Fig. 3). For example, the second patriline in colony 1 (pink) and the 4th patriline in colony 5 (dark purple) produced substantially more gynes than workers, proportionally. Thus, these patrilines could be viewed as evolutionary “winners”, whereas other patrilines might be viewed as “losers”. Surprisingly, there was 1 patriline (7th, gray) in colony 1 that produced only workers and no gynes (Table 2 and Fig. 3). Overall, our data shows clear evidence for modest levels of caste-biased paternity skew.

Few previous studies have investigated differential contributions to sexual versus sterile offspring in colonial insect species. Patriline competition for producing gynes has been documented in the ant, *Formica sanguinea* (Pamilo & Seppä, 1994; Haapaniemi & Pamilo, 2012). Similarly, about 20% of patrilines were found to be biased toward gyne production in the leaf cutter ant, *Acromyrmex echinator* (Hughes & Boomsma, 2008). Caste-biased paternity skew also has been documented in honeybees, *Apis mellifera* (Moritz *et al.*, 2005). In social wasps, there has been little evidence for caste-biased paternity skew previously (Goodisman *et al.*, 2007a). Overall, it is unclear how many social insect taxa may experience caste-biased paternity skew and this should be a prioritized area of research in the future.

The mechanisms by which males may end up siring different proportions of workers and gynes in *V. maculifrons* are unclear. *V. maculifrons* queens could select the sperm of certain males to fertilize certain eggs (Eberhard, 1996). That is, a queen might purposefully over-utilize sperm from 1 male that appears to be particularly fit for the majority of her reproductive offspring (Simmons, 2001). If this prediction is true, it may explain why we did not find a phenotypic tradeoff for offspring of high-paternity males. Further, queens may also physiologically govern



which male's sperm fertilizes eggs destined for a particular caste. Overall, such female choice for preferential sperm use could be an important mechanism governing male reproductive success.

Finally, there may be stochastic elements to male success in worker versus gyne production. *V. maculifrons* follows an annual life cycle and gynes are produced at the end of the season. Thus, any mechanism of male precedence that might operate in *V. maculifrons* whereby, for example, males that mated with queens first also were most likely to have their sperm used last (i.e., first in, last out male precedence), might lead to the observed patterns (Johnson *et al.*, 2009). Such mechanisms of male precedence are difficult to test for in unmanaged species such as *V. maculifrons* but are likely to be of importance in many taxa.

#### *Patterns of paternity skew over time*

Reproductive behaviors may be strongly labile and subject to both evolutionary change arising from the pressures of natural selection or plastic responses arising from shifting environmental conditions. Examining changes in reproductive behaviors over time can thus be informative in understanding the evolution and ecology of reproductive activities. Therefore, we compared our data on paternity skew and queen mate number to similar data obtained from prior studies in this species and geographical region from 1984, 2004, 2006, and 2017 (Ross, 1986; Goodisman *et al.*, 2007b; Kovacs *et al.*, 2008; Dyson *et al.*, 2021). We were particularly interested in determining if mating patterns and paternity skew had changed in light of ongoing climate variability.

We found no evidence of changes in queen mate number or effective mate number over time in our study (Fig. 4B). Instead, these values remained remarkably similar over the past ~40 years. This finding reveals the extraordinary stability of polyandrous mating strategy in this species over time. In addition, there were no significant differences in the magnitude of paternity skew across years 2004–2022. Although this study did identify significant skew, unlike previous investigations, the magnitude of skew was similar across years.

Thus, we have no evidence of changes in paternity behaviors in this species; instead queen mate number and male skew seem remarkably stable over time. *V. maculifrons* queens target a fairly tight range of intermediate mate number (Table 2 and Fig. 4). None of the queens sampled in our study mated with fewer than 5 or more than 8 males. So, this narrow range in mate number would seem to be of importance in this species.

#### *Paternity skew and evolutionary trade-offs*

We tested evolutionary trade-off theory by investigating if male paternity skew was correlated with offspring phenotype. Theoretically, genotypes with strong caste effects (i.e., “cheaters” which produce many offspring) are expected to be held at low frequency (Hughes & Boomsma, 2008; Van Dyken *et al.*, 2011). That is, genotypes that show “cheating” in social behaviors should be selected against through kin-selected processes. Thus, such cheating genotypes may invade transiently, but they will be selected against by other evolutionary processes and should never reach particularly high frequency in a population.

We investigated one interpretation of this hypothesis by examining if males that were strongly successful in terms of reproductive success also suffered some trade-offs in terms of the fitness of offspring they produced. We suggested that one such trade-off would be the production of offspring of small body size. In particular, selection should act strongly on body size in gynes. Social insects whose queens found colonies independently, as is the case in *V. maculifrons*, are selected for large body size (Kingsolver & Pfennig, 2004; Prešern & Smodiš Škerl, 2019). Queen body mass plays an important role in overwintering success and future colony health (Holm, 1972; Kovacs & Goodisman, 2012; Keaveny & Dillon, 2022), and, therefore, gynes are expected to be selected for large body size. Thus, we proposed that males who achieved high overall reproductive success (i.e., producing a large number of both workers and gynes) might suffer some counterbalancing selective disadvantage in terms of the quality of offspring they produced. Specifically, we hypothesized that gynes sired by a male who produced many offspring, may develop into a less desirable morphological phenotype.

We found that only 2 traits, forewing length and resilin joint area length, was significantly different in gyne castes from different patriline (Fig. 5 and Table S2). Specifically, less frequent patriline produced gyne daughters with longer forewings and longer resilin joint areas on the forewings. Interestingly, patriline effects on wing morphology in *Vespula* wasps have been noted previously (Perrard & Loope, 2015). This may indicate an important genetic basis to wing morphology in social wasps. Regardless, the observed relationship in our study was weak and uncovered in only these 2 traits. So, the general lack of patriline effects on morphology suggests that there are no clear reproductive “trade-offs” for *V. maculifrons* males.

Importantly, our test of this hypothesis was indirect because male skew observed in our study may not be

associated consistently with genotypic effects. Instead, the finding that there were no morphological differences between patrines suggests that paternity skew may, in fact, be incidentally related to sperm usage over time rather than result from genetic effects on developmental pathways. Moreover, the size of offspring may not always be fitness-associated. Or there may be other fitness related traits correlated with male reproductive success which were unmeasured in this study.

## Conclusions

Polyandry is abundant in nature and can have important implications for reproductive competition and relatedness within families. Here, we explored how polyandry influenced reproductive outcomes in a highly social wasp. Our study uncovered significant paternity skew and caste-biased skew in the Eastern yellowjacket wasp, *V. maculifrons*. Moreover, we found little evidence for changes in mating patterns of queens over ~40 years of investigation. We also failed to document strong trade-offs between male reproductive success and offspring quality. We posit that future studies should explore phenotypic differences in offspring from taxa with more extreme reproductive skew. This could help us better understand the consequences of polyandry in nature. Overall, our study provides important information on paternity skew and competition in highly social insects.

## Acknowledgments

Research funding provided by NSF-DEB: 2105033 and NSF-IOB: 2019799 to Michael A. D. Goodisman and USDA: 2023-67012-39886 to Sarah E. Orr. We would like to thank Paige Caine and Andrew Robertson for their assistance with collecting the wasp colonies.

## Disclosure

The authors declare no potential conflicts of interest.

## References

- Alattal, Y., Al-Ghamdi, A., Alsharhi, D.M. and Fuchs, S. (2014) Morphometric characterisation of the native Honeybee, *Apis mellifera* Linnaeus, 1758, of Saudi Arabia. *Zoology in the Middle East*, 60, 226–235.
- Allard, D., Gobin, B. and Billen, J. (2007) Timing of sperm transfer in *Diacamma pallidum*. *Physiological Entomology*, 32, 382–387.
- Baer, B. (2011) The copulation biology of ants (Hymenoptera: Formicidae). *Myrmecol News*, 14, 55–68.
- Baer, B. (2014) Sexual selection in social insects. In: *The Evolution of Insect Mating Systems* (eds. D. Shuker & L. Simmons), pp. 261–274. Oxford University Press.
- Baer, B. (2015) Female choice in social insects. In: *Cryptic Female Choice in Arthropods: Patterns, Mechanisms and Prospects* (eds. A.V. Peretti & A. Aisenberg), pp. 461–477. Springer International Publishing, Cham.
- Baer, B. (2016) Proximate and ultimate consequences of polyandry in ants (Hymenoptera: Formicidae). *Myrmecological News*, 22, 1–9.
- Baer, B. and Schmid-Hempel, P. (2005) Sperm influences female hibernation success, survival and fitness in the bumblebee *Bombus terrestris*. *Proceedings of the Royal Society B*, 272, 319–323.
- Baer, B., Schmid-Hempel, P., Høeg, J.T. and Boomsma, J.J. (2003) Sperm length, sperm storage and mating system characteristics in bumblebees. *Insectes Sociaux*, 50, 101–108.
- Bonckaert, W., Vuerinckx, K., Billen, J., Hammond, R.L., Keller, L. and Wenseleers, T. (2008) Worker policing in the German wasp *Vespula germanica*. *Behavioral Ecology*, 19, 272–278.
- Boomsma, J.J. (2013) Beyond promiscuity: mate-choice commitments in social breeding. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368, 20120050.
- Boomsma, J.J. and Ratnieks, F.L.W. (1997) Paternity in eusocial Hymenoptera. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences*, 351, 947–975.
- Boulton, R.A. and Shuker, D.M. (2013) Polyandry. *Current Biology*, 23, R1080–R1081.
- Boulton, R.A., Zuk, M. and Shuker, D.M. (2018) An inconvenient truth: the unconsidered benefits of convenience polyandry. *Trends in Ecology & Evolution*, 33, 904–915.
- Daly, D., Archer, M.E., Watts, P.C., Speed, M.P., Hughes, M.R., Barker, F.S. et al. (2002) Polymorphic microsatellite loci for eusocial wasps (Hymenoptera: Vespidae). *Molecular Ecology Notes*, 2, 273–275.
- Davies, N.G. and Gardner, A. (2018) Monogamy promotes altruistic sterility in insect societies. *Royal Society Open Science*, 5, 172190.
- Degueldre, F. and Aron, S. (2023) Long-term sperm storage in eusocial Hymenoptera. *Biological Reviews*, 98, 567–583.
- den Boer, S.P.A., Baer, B. and Boomsma, J.J. (2010) Seminal fluid mediates ejaculate competition in social insects. *Science*, 327, 1506–1509.
- Dillard, J.R. and Westneat, D.F. (2016) Disentangling the correlated evolution of monogamy and cooperation. *Trends in Ecology & Evolution*, 31, 503–513.
- Dyson, C.J., Crossley, H.G., Ray, C.H. and Goodisman, M.A.D. (2022) Social structure of perennial *Vespula squamosa* wasp colonies. *Ecology and Evolution*, 12, e8569.

- Dyson, C.J., Piscano, O.L., Durham, R.M., Thompson, V.J., Johnson, C.H. and Goodisman, M.A.D. (2021) Temporal analysis of effective population size and mating system in a social wasp. *Journal of Heredity*, 112, 626–634.
- Eberhard, W. (1996) *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton University Press.
- Forfert, N., Troxler, A., Retschnig, G., Gauthier, L., Straub, L., Moritz, R.F.A. *et al.* (2017) Neonicotinoid pesticides can reduce honeybee colony genetic diversity. *PLoS ONE*, 12, e0186109.
- Foster, K.R. and Ratnieks, F.L. (2001) Paternity, reproduction and conflict in vespine wasps: a model system for testing kin selection predictions. *Behavioral Ecology and Sociobiology*, 50, 1–8.
- Foster, K.R., Ratnieks, F.L.W., Gyllenstrand, N. and Thorén, P.A. (2001) Colony kin structure and male production in *Dolichovespula* wasps. *Molecular Ecology*, 10, 1003–1010.
- Fromonteil, S., Marie-Orleach, L., Winkler, L. and Janicke, T. (2023) Sexual selection in females and the evolution of polyandry. *PLoS Biology*, 21, e3001916.
- Goodisman, M.A.D., Kovacs, J.L. and Hoffman, E.A. (2007a) Lack of conflict during queen production in the social wasp *Vespula maculifrons*. *Molecular Ecology*, 16, 2589–2595.
- Goodisman, M.A.D., Kovacs, J.L. and Hoffman, E.A. (2007b) The significance of multiple mating in the social wasp *Vespula maculifrons*. *Evolution; International Journal of Organic Evolution*, 61, 2260–2267.
- Goodisman, M.A.D., Matthews, R.W. and Crozier, R.H. (2002) Mating and reproduction in the wasp *Vespula germanica*. *Behavioral Ecology and Sociobiology*, 51, 497–502.
- Haapaniemi, K. and Pamilo, P. (2012) Reproductive conflicts in polyandrous and polygynous ant *Formica sanguinea*. *Molecular Ecology*, 21, 421–430.
- Hammond, R.L. and Keller, L. (2004) Conflict over male parentage in social insects. *PLoS Biology*, 2, e248.
- Hannonen, M. and Sundström, L. (2003) Worker nepotism among polygynous ants. *Nature*, 421, 910.
- Hare, R.M. and Simmons, L.W. (2019) Sexual selection and its evolutionary consequences in female animals. *Biological Reviews*, 94, 929–956.
- Hasegawa, E. and Takahashi, J. (2002) Microsatellite loci for genetic research in the hornet *Vespa mandarinia* and related species. *Molecular Ecology Notes*, 2, 306–308.
- Hoffman, E.A., Kovacs, J.L. and Goodisman, M.A. (2008) Genetic structure and breeding system in a social wasp and its social parasite. *BMC Evolutionary Biology*, 8, 239.
- Holm, S.N. (1972) Weight and life length of hibernating bumble bee queens (Hymenoptera: Bombidae) under controlled conditions. *Insect Systematics & Evolution*, 3, 313–320.
- Holzer, B., Chapuisat, M., Kremer, N., Finet, C. and Keller, L. (2006) Unicoloniality, recognition and genetic differentiation in a native *Formica* ant. *Journal of Evolutionary Biology*, 19, 2031–2039.
- Hughes, W.O.H. and Boomsma, J.J. (2008) Genetic royal cheats in leaf-cutting ant societies. *Proceedings of the National Academy of Sciences*, 105, 5150–5153.
- Hunt, J.H. and Toth, A.L. (2017) Sociality in Wasps. In: *Comparative Social Evolution* (eds D.R. Rubenstein & P. Abbot), pp. 84–123. Cambridge University Press: Cambridge.
- Jaffé, R. (2014) An updated guide to study polyandry in social insects. *Sociobiology*, 61, 1–8.
- Jaffé, R., Garcia-Gonzalez, F., den Boer, S.P.A., Simmons, L.W. and Baer, B. (2012) Patterns of paternity skew among polyandrous social insects: what can they tell us about the potential for sexual selection? *Evolution; International Journal of Organic Evolution*, 66, 3778–3788.
- Johnson, E.L., Cunningham, T.W., Marriner, S.M., Kovacs, J.L., Hunt, B.G., Bhakta, D.B. *et al.* (2009) Resource allocation in a social wasp: effects of breeding system and life cycle on reproductive decisions. *Molecular Ecology*, 18, 2908–2920.
- Keaveny, E.C. and Dillon, M.E. (2022) That queens emerge fashionably late: body size and condition predict timing of spring emergence for queen bumble bees. *Insects*, 13, 870.
- Kingsolver, J.G. and Pfennig, D.W. (2004) Individual-level selection as a cause of cope's rule of phyletic size increase. *Evolution; International Journal of Organic Evolution*, 58, 1608–1612.
- Kovacs, J.L. and Goodisman, M.A.D. (2012) Effects of size, shape, genotype, and mating status on queen overwintering survival in the social wasp *Vespula maculifrons*. *Environmental Entomology*, 41, 1612–1620.
- Kovacs, J.L., Hoffman, E.A. and Goodisman, M.A.D. (2008) Mating success in the polyandrous social wasp *Vespula maculifrons*. *Ethology*, 114, 340–350.
- Kovacs, J.L., Hoffman, E.A., Marriner, S.M. and Goodisman, M.A.D. (2010) Detecting selection on morphological traits in social insect castes: the case of the social wasp *Vespula maculifrons*. *Biological Journal of the Linnean Society*, 101, 93–102.
- Loope, K.J., Chien, C. and Juhl, M. (2014) Colony size is linked to paternity frequency and paternity skew in yellowjacket wasps and hornets. *BMC Evolutionary Biology*, 14, 277.
- Mattila, H.R. and Seeley, T.D. (2007) Genetic diversity in honey bee colonies enhances productivity and fitness. *Science*, 317, 362–364.
- Moritz, R.F.A., Lattorff, H.M.G., Neumann, P., Kraus, F.B., Radloff, S.E. and Hepburn, H.R. (2005) Rare royal families in honeybees, *Apis mellifera*. *Die Naturwissenschaften*, 92, 488–491.
- Nielsen, R., Tarpy, D.R. and Reeve, H.K. (2003) Estimating effective paternity number in social insects and the effective number of alleles in a population. *Molecular Ecology*, 12, 3157–3164.

- Nonacs, P. (2000) Measuring and using skew in the study of social behavior and evolution. *The American Naturalist*, 156, 577–589.
- Nonacs, P. and Hager, R. (2011) The past, present and future of reproductive skew theory and experiments. *Biological Reviews Cambridge Philosophical Society*, 86, 271–298.
- Pamilo, P. and Seppä, P. (1994) Reproductive competition and conflicts in colonies of the ant *Formica sanguinea*. *Animal Behaviour*, 48, 1201–1206.
- Perrard, A. and Loope, K.J. (2015) Patriline differences reveal genetic influence on forewing size and shape in a yellow-jacket wasp (Hymenoptera: Vespidae: *Vespula flavopilosa* Jacobson, 1978). *PLoS ONE*, 10, e0130064.
- Pizzari, T. and Wedell, N. (2013) The polyandry revolution. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368, 20120041.
- Prešern, J. and Smodiš Škerl, M.I. (2019) Parameters influencing queen body mass and their importance as determined by machine learning in honey bees (*Apis mellifera carnica*). *Api-dologie*, 50, 745–757.
- Radloff, S.E., Hepburn, H.R., Hepburn, C., Fuchs, S., Otis, G.W., Sein, M.M. et al. (2005) Multivariate morphometric analysis of *Apis cerana* of southern mainland Asia. *Apidologie*, 36, 127–139.
- Radloff, S.E., Hepburn, R., Neumann, P., Moritz, R.F.A. and Kryger, P. (2002) A method for estimating variation in the phenotypic expression of morphological characters by thelytokous parthenogenesis in *Apis mellifera capensis*. *Apidologie*, 33, 129–137.
- Ross, K. (1986) Kin selection and the problem of sperm utilization in social insect. *Nature*, 323, 798–800.
- Ross, K.G. and Matthews, R.W. (1991) *The Social Biology of Wasps*. Cornell University Press.
- Ruttner, F., Tassencourt, L. and Louveaux, J. (1978) Biometrical-statistical analysis of the geographic variability of *Apis mellifera* L. I. material and methods. *Apidologie*, 9, 363–381.
- Saga, T., Okuno, M., Loope, K.J., Tsuchida, K., Ohbayashi, K., Shimada, M. et al. (2020) Polyandry and paternity affect disease resistance in eusocial wasps. *Behavioral Ecology*, 31, 1172–1179.
- Schlüns, H., Moritz, R., Lattorff, H.M. and Koeniger, G. (2005) Paternity skew in seven species of honeybees (Hymenoptera: Apidae: Apis). *Apidologie*, 36, 201–209.
- Seeley, T.D. and Tarpy, D.R. (2007) Queen promiscuity lowers disease within honeybee colonies. *Proceedings of the Royal Society*, 274, 67–72.
- Shuker, D. and Simmons, L. (2014) *The Evolution of Insect Mating Systems*. Oxford University Press.
- Simmons, L.W. (2001) *Sperm Competition and Its Evolutionary Consequences in the Insects*, Princeton University Press.
- Simmons, L.W. and García-González, F. (2008) Evolutionary reduction in testes size and competitive fertilization success in response to the experimental removal of sexual selection in dung beetles. *Evolution; International Journal of Organic Evolution*, 62, 2580–2591.
- Simmons, L.W. and Garcia-Gonzalez, F. (2011) Experimental coevolution of male and female genital morphology. *Nature Communications*, 2, 374.
- Simmons, L.W. and Wedell, N. (2020) Fifty years of sperm competition: the structure of a scientific revolution. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 375, 20200060.
- Snook, R.R. (2014) The evolution of polyandry. In *The Evolution of Insect Mating Systems* (eds. D. Shuker & L. Simmons), pp. 159–180. Oxford University Press.
- Spradbery, J.P. (1973) Wasps: an account of the biology and natural history of solitary and social wasps. *Bulletin of the Entomological Society of America*, 20, 335–336.
- Sundström, L. and Boomsma, J.J. (2000) Reproductive alliances and posthumous fitness enhancement in male ants. *Proceedings of the Royal Society of London Series B: Biological Sciences*, 267, 1439–1444.
- Tarpy, D.R., Delaney, D.A. and Seeley, T.D. (2015) Mating frequencies of honey bee queens (*Apis mellifera* L.) in a population of feral colonies in the Northeastern United States. *PLoS ONE*, 10, e0118734.
- Tarpy, D.R. and Nielsen, D. (2002) Sampling error, effective paternity, and estimating the genetic structure of honey bee colonies (Hymenoptera: Apidae). *Annals of the Entomological Society of America*, 95, 513–528.
- Taylor, D., Bentley, M.A. and Sumner, S. (2018) Social wasps as models to study the major evolutionary transition to super-organismality. *Current Opinion in Insect Science*, 28, 26–32.
- Taylor, M.L., Price, T.A.R. and Wedell, N. (2014) Polyandry in nature: a global analysis. *Trends in Ecology & Evolution*, 29, 376–383.
- Van Dyken, J.D., Linksvayer, T.A. and Wade, M.J. (2011) Kin selection–mutation balance: a model for the origin, maintenance, and consequences of social cheating. *The American Naturalist*, 177, 288–300.
- Walsh, P.S., Metzger, D.A. and Higuchi, R. (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques*, 10, 506–513.
- Wenseleers, T., Badcock, N.S., Erven, K., Tofilski, A., Nascimento, F.S., Hart, A.G. et al. (2005) A test of worker policing theory in an advanced eusocial wasp, *Vespula rufa*. *Evolution; International Journal of Organic Evolution*, 59, 1306–1314.
- Wiernasz, D.C. and Cole, B.J. (2003) Queen size mediates queen survival and colony fitness in harvester ants.

*Evolution; International Journal of Organic Evolution*, 57, 2179–2183.

Wiernasz, D.C. and Cole, B.J. (2010) Patriline shifting leads to apparent genetic caste determination in harvester ants. *Proceedings of the National Academy of Sciences USA*, 107, 12958–12962.

Wright, C.M., Lichtenstein, J.L.L., Tibbetts, E.A. and Pruitt, J.N. (2019) Individual variation in queen morphology and behavior predicts colony performance in the wild. *Behavioral Ecology and Sociobiology*, 73, 122.

Manuscript received November 9, 2023

Final version received January 22, 2024

Accepted January 24, 2024

## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1** Technical information for the 6 microsatellite markers used to genotype wasps in this study.

**Table S2** F ratio and significance values (*P*) for patriline effects for 8 morphological traits in gynes and workers.