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Maternal and paternal influences on mating frequency in harvester ants

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Multiple mating by females is taxonomically widespread and intensively studied from the perspective of why females mate with many males. In many multiply mating species, females can vary substantially in mating frequency, but the causes of this variation are not well understood. We used directed mating to explore the causes of variation in mating frequency in a harvester ant whose queens mate an average of 10 times but where naturally occurring mating frequency ranges from 2 to 15 mates. Matrilines differed in mating frequency and especially in their probability of mating with the first male lineage that they encountered. Differences in matriline mating frequency were not related to differences in female size among matrilines. Male mating success was not correlated with the order in which males encountered females, suggesting that male success may depend on which matrilines they encounter. Our results suggest that variation in mating frequency may be a consequence of differences among matrilines due to additive genetic and/or maternal effects, as has been found in other species.

Multiple mating by females is now recognized to occur in all major taxonomic groups (Parker & Birkhead, 2013). Females may benefit from multiple mates because the males provide direct benefits that increase female survival or fecundity (Arnqvist & Nilsson, 2000). If they cannot discern male quality in advance, multiply mating females may benefit indirectly through sperm competition or cryptic female choice (Eberhard, 1996; Parker, 1970), leading to higher-quality offspring (although the data are mixed; reviewed in Slayter, Mautz, Backwell, & Jennions, 2012). Multiple mating will increase the genetic diversity among a female's offspring, which may lead to greater reproductive success (Jennions & Petrie, 2000). Multiple mating may also enable females to avoid the costs of mating with an incompatible male, leading to reproductive failure (Zeh & Zeh, 1997).

Despite these benefits, females of many species display considerable variation in mating frequency in the field (beetles: Haddrill, Shuker, Amos, Majerus, & Mayes, 2008; Miyatake & Matsumura, 2004; butterflies: Bergström, Wiklund, & Kaitala, 2002; Burns, 1968; crickets: Bretman & Tregenza, 2005; Rodríguez-Muñoz, Bretman, Slate, Walling, & Tregenza, 2010; Simmons, Beveridge, & Kennington, 2007; *Drosophila*: Price, Lewis, Smith, Hurst, & Wedell, 2011; dungflies: Demont, Buser, Martin, & Bussière, 2011; Demont, Martin, & Bussière, 2012; guppies: Evans & Gasparini, 2013; moths: McNamara, Elgar, & Jones, 2008). The causes of this variation are unclear. Females may be constrained from achieving their optimum mating frequency. The operational sex ratio (McNamara et al., 2008), overall population density (Carillo, 2007; Välimäki & Kaitala, 2006) and relative protandry (female emergence relative to males; Rhainds, 2012) have all been shown to cause variation in female mating frequency. Female attractiveness, including variation in body size (Bergström et al., 2002; McNamara et al., 2008) and age (Kwon, Amin, & Suh, 2006), may also contribute to this variation. In such cases, we expect increased mating frequency to be positively correlated with female fitness (i.e. directional selection for mating frequency).

Alternatively, variation may be a result of sexual conflict where some females mate beyond the optimum value. Conflict over mating frequency may be common because male reproductive success typically increases with higher mating frequency (Arnold & Duvall, 1994), while that of females may not. Females often incur substantial costs by resisting additional mating, including increased risk of predation, higher energetic costs and higher risk of injury or death (reviewed in Arnqvist & Rowe, 2005). If levels of male harassment and thus costs are sufficiently high, females may mate more frequently to reduce these costs, leading to

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'convenience polyandry' (Arnqvist, 1989; Panova et al., 2010). Finally, variation in female mating frequency may be a consequence of chance or other random effects, especially when the costs of mating are low (Bleu, Bessa-Gomes, & Laloi, 2012; Kokko & Mappes, 2012; Tarpy & Page, 2000).

Variation in mating frequency will also arise when females vary in the shape of their mate preference function or in their degree of choosiness, both of which may be influenced by female age, size or experience (Kwon et al., 2006; Liedo, De Leon, Barrios, Valle-Mora, & Ibarra, 2002; Marie-Orleach, Janicke, & Schärer, 2013; Price et al., 2011). A growing body of research suggests that intrinsic differences among females underlie differences in mating frequency (Harano & Miyatake, 2005; Kraus, Neumann, & Moritz, 2005; Pyle & Gromko, 1979; Shuker, Phillimore, Burton-Chellew, Hodge, & West, 2007; Simmons, 2003; Torres-Vila, Gragera, Rodriguez-Molina, & Stockel, 2002; Torres-Vila, Rodriguez-Molina, Gragera, & Bielza-Lino, 2001; Wedell, Wiklund, & Cook, 2002).

In social insects, high levels of multiple mating by queens are restricted to a few groups, including *Apis* bees (e.g. Éstoup, Solignac, & Cornuet, 1994), vespulid wasps (e.g. Foster & Ratnieks, 2001), fungus-growing ants (e.g. Boomsma, Fjerdingstad, & Frydenberg, 1999), army ants (e.g. Kronauer, Schöning, Pedersen, Boomsma, & Gadau, 2004) and harvester ants (Rheindt, Gadau, Strehl, & Hölldobler, 2004; Wiernasz, Perroni, & Cole, 2004). The main selective advantage to multiple mating in these species has been the advantage of a genetically diverse worker force, which leads to greater resistance to disease (Brown & Schmid-Hempel, 2003; Seeley & Tarpy, 2007), more effective division of labour (Oldroyd & Fewell, 2007) and higher colony performance (Cole & Wiernasz, 1999; Mattila & Seeley, 2007). Natural populations of species whose queens mate with many males vary considerably in the number of patrilines (male genotypes) present in the colony, indicative of variation in mating frequency. The degree to which females control the number of males that they mate with is unknown for any species, although Tarpy and Page (2000) suggested that variation in mating frequency of *Apis mellifera* queens may arise largely by chance.

We used controlled matings to explore the causes of variation in female mating propensity in the western harvester ant, *Pogonomyrmex occidentalis* Cresson. Reproductives of *P. occidentalis* mate in large swarms with a strongly male-biased operational sex ratio (Abell, Cole, Reyes, & Wiernasz, 1999). Swarms occur on locally prominent hilltops, usually on a single day during the summer. Reproductive flights are triggered by rainfall and typically occur after the onset of the monsoon season in early to mid-July. After mating, queens fly to the desert floor to initiate colonies, while males remain at the swarm sites. Colonies are headed by a single, multiply mated queen and are initiated immediately after the mating flight. Queens mate only on the day of the reproductive flight and may store sperm for decades.

In this paper we tested the hypothesis that variation in mating frequency is a consequence of differences among matrilines. We predicted that matrilines would vary both in the total number of times they mated and in their propensity to mate (how readily they mated with the first males they encountered). We also tested the hypothesis that male lineages differ in mating vigour, leading to differences in male mating success.

METHODS

Directed matings have been used previously in several species of ants to understand the mechanics of sperm transfer (Allard, Gobin, Ito, Tsuji, & Billen, 2002; Allard et al., 2006; Oppelt & Heinze, 2007; Robertson, 1995), to explore multiple mating by males (Allard, Van Hulle, Billen, & Gobin, 2008), to explore consequences of mating on female life span (Schrempf, Heinze, & Cremer, 2005), including the effects of mating with different male morphs (Schrempf & Heinze, 2008), and to examine genetic propensity for caste determination (Libbrecht, Schwander, & Keller, 2011; Schwander & Keller, 2008). In most of these studies, females were mated to a single male. We sequentially exposed females to groups of males from specific colonies to examine the relative effect of male and female genotype on mating frequency.

The directed matings took place at our long-term study site (Wiernasz & Cole, 1995). Excavation of nests indicates that fully pigmented reproductives may be found as early as mid-June, depending on the year. Our studies of reproductive allocation (Cole & Wiernasz, 2000) are usually conducted in early July before the onset of the summer rains. We assume that gynes and males that are 'flight-ready' are also reproductively competent. Our overall approach was to sequentially mass-mate reproductives from specific colonies to produce colonies that could be reared through their initial growth stages in the laboratory. The identity and number of mates were determined by sampling the resulting workers.

To stimulate the flight of reproductives, we applied water to the colonies. Approximately 6–8 litres of water was placed on 8–12 colonies on 1 day to induce the flight of reproductives on the next afternoon (Cole & Wiernasz, 2000). Reproductively mature colonies of *P. occidentalis* do not reproduce every year. Consequently, we watered an excess number of colonies, anticipating that some would not reproduce. The number of colonies available for crosses ranged from 4 to 10; on most days, the colonies that yielded males, also produced females. However, on two days, we obtained gynes from four colonies but males from only three. When reproductives begin to emerge from the colony in advance of flying, the gynes are the first to emerge, presumably to achieve the high body temperature required for flight. We collected 5–10 gynes from each colony, which were held in vials in the shade but not chilled. After collecting the gynes, we placed reproductive traps over the nests, in order to collect males from the same colonies. We harvested males when we estimated that there were 200–300 males in the trap. Males were placed in 8 litre plastic bags and chilled for 10–15 min in a cooler. Male collections were checked to ensure that no gynes were present; if gynes were found, they were removed. Because these gynes may have mated within the trap, they could not be used in experiments. They were kept in a cooler during the afternoon and later frozen.

During a mating trial, the 5–10 gynes from one female source colony all were placed with 200–300 males collected from a different colony in a 1.5-litre plastic tub covered with fine fabric mesh. We were able to set up as many as eight mating trials at once. The containers were placed in the sun, but off the hot ground, for 20 min, during which the containers were monitored to ensure that the reproductives did not overheat. If a significant fraction (~one-third) of the males were on the mesh top rather than in the bottom of the tub, it was moved briefly (2–3 min) into the shade and then back out into the sun. Instances of potential overheating were rare, happening fewer than 10 times during the experiment. After 20 min, the tubs were chilled briefly ($\leq$5 min) in a cooler, and all gynes were removed and placed into another tub with males from a different colony. Males were left in their original tub. Gynes from each colony were placed successively with males from up to four different colonies (weather truncated the experiment on 2 days). All crosses were made on the day of the induced mating flight. We controlled the access that a gyne had to males from a colony, but we could not ensure that she mated with a male from each colony. Gynes could potentially mate with multiple males (brothers) from the same colony. We repeated this procedure for 6 days and obtained mated queens from a total of 39 gyne source colonies (matrilines) using males from 36 colonies. After the sixth day, rain

caused a natural reproductive flight, making it impossible to continue the experiments.

On two of the days that we carried out controlled matings, we collected additional gynes from some colonies. These females were presented with members of several male lineages simultaneously rather than sequentially. A single gyne was placed in a 0.2-litre plastic tub with two males from each of up to seven other colonies ('batch' matings) and allowed to mate for 30 min. Males belonging to a gyne's colony were not used in these trials. We used this method in case females mated indiscriminately with the first group of males and completed all of their mating within 20 min. Mated foundresses were placed in test-tubes half-filled with water and plugged with cotton. They were held for 3 days at 25 °C before being shipped overnight to the University of Houston.

Each mated queen was allowed to found a colony in the laboratory. Colonies were grown in the laboratory in plastic boxes with a food source (50% honey-water solution in Kimpak® packing material; organic sunflower seeds and cracked wheat; cut up cricket pieces). The boxes were housed in an incubator at 28 °C in constant darkness. New food was added weekly and unused food was removed. The queen cares for the brood until the first workers emerge, which takes approximately 4 weeks. In the benign conditions of the laboratory, colonies may reach a size of 100 workers in as little as 5 months. After approximately 8 months, we removed a sample of 18 workers for genetic analysis. Early on, sperm from different males may not be homogeneously distributed within the spermatheca, and worker genotypes may not represent all males that have mated with the queen. In honeybees, worker genotype distributions and spermathecal genotype distributions become progressively more similar over the first 6 months postmating (Franck et al., 2002). Consequently, early samples may underestimate the total number of times that a queen has mated. To increase the probability that we detected all matings that had occurred, we chose workers with different degrees of cuticular pigmentation in order to sample a range of worker ages. We also collected a second sample of workers at approximately 16 months, using the same criteria as before. If a colony's queen died, but there were sufficient workers for a sample, workers from these colonies were genotyped. Data from the two worker samples were combined and the maximum number of detected matings was used to determine mating frequency.

Genotyping was performed using four highly variable microsatellite loci developed for *P. occidentalis* (with 28, 19, 15 and 12 alleles; range of heterozygosities: 0.81–0.97; for details of DNA extraction and PCR, see Wiernasz et al., 2004). PCR products were labelled internally using a fluorescent marker (5-FAM, HEX or NED®) and separated on an ABI 3730 genetic analyser at the Arizona State University core facility. Allele identities were assigned using the ABI Peakscanner program. In haplodiploid organisms such as ants, genotyping diploid offspring readily identifies the genoytpes of the diploid (female) parent, and all daughters of the same father will share an identical multilocus genotype (patriline). All source colonies used in the experiments had been genotyped previously. The queen's genotype and patriline identities were determined by inspection and confirmed by comparison to genotypes identified for the male and female source colonies. We set up the controlled matings so that the multilocus genotype of each male lineage was distinct from all other male lineages that had potentially mated with the queens of a particular matriline. Although we could distinguish patrilines with complete certainty, there is a small but nonzero probability that a gyne could mate with two brothers of identical genotype (on average, $P = 0.0625$), thus we may have underestimated the actual mating frequency slightly. The presence of a particular male lineage among worker genotypes was confirmation that the queen had mated with that lineage. Mating frequency was estimated as the total number of unique patrilines detected in the pooled samples of workers. We did not genotype the spermathecal contents of the experimental queens, so we could not distinguish male lineages that mated with a female but whose sperm was not used versus male lineages that did not mate.

All experimental ants were handled carefully throughout the course of this work. We took care while ants were being mated to ensure that they did not overheat or dessicate. After each day's experiment, all males were frozen. Ants collected during the course of rearing were also immediately frozen. All ants in colonies that were terminated because they did not produce brood, as well as from any colony whose queen died prematurely, were also frozen.

We could not measure queen mass directly in this study because we did not have access to a sensitive balance in the field. Measurements of fresh mass that could be made later would have additional variation due to queen hydration and oviposition. We estimated queen mass for a matriline from the historical average dry mass of queens produced by these colonies (based on 3–4 years of reproductive data for each colony in question). Average dry mass of gynes is quite consistent for a given colony. For individual samples of at least 50 gynes, the coefficient of variation for dry mass ranged from 0.04 to 0.26 (median = 0.087; 138 of 199 samples collected over 4 years had CVs $\leq$0.10, Cole & Wiernasz, n. d.). Across years, the mean colony CV was 0.039, with a range of 0.012–0.109, $N = 35$ colonies (Cole & Wiernasz, n.d.). We used the average dry mass and the average mating frequency for a matriline to test for a positive correlation between gyne size and mating frequency.

We used ANOVA to determine whether matrilines differed in mating frequency and in the tendency to mate with the first males they encountered. A queen's mating frequency was the number of different patrilines identified from the pooled worker samples. The proportion of total matings by a queen that were with the first males presented was arcsine square-root transformed before analysis. The proportion of gynes mating with the first males may be a consequence of male lineage, female lineage, or an interaction between the two. To fully dissect this, we would have needed to place females from the same lineage with males from the same lineages but in a different order. Logistically, this experimental structure was not feasible because of the limited number of gynes that could be harvested from a colony before reproductives began to fly. Variation in male lineage mating success during the first mating opportunity is not independent of female lineage effects. We used two approaches to ask whether male lineages differed in mating success. If mating success is a property of a lineage, males should be successful regardless of what female lineage they are placed with. Male success was defined as the fraction of possible matings that were obtained by a male lineage, based on the total number of available matings by that matriline. For example, if the females of a matriline collectively mated a total of 36 times, and 12 of those matings were with the first male lineage that they were paired with, the proportion of available first matings for that male lineage would be 12/36. If the next group of females that this male lineage was paired with mated a total of 18 times with all males after the first group (i.e. all second, third and fourth males), and the male lineage achieved five matings, the proportion of available second matings would be 5/18. We tested the correlation between first and second mating success across all male lineages. We measured residual male mating success (all mating opportunities after the first) for each male lineage by comparing the number of successful and unsuccessful subsequent matings, corrected for the number of available matings. We used a G test of heterogeneity to determine the success of males on each day of the experiment and pooled across all days. All analyses were carried out in SYSTAT 11 (Wilkinson, 2004).

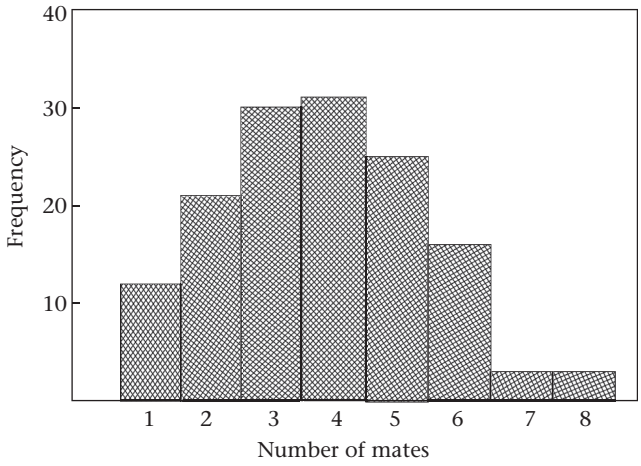

Figure 1. Distribution of mating frequencies for all colonies. Number of mates was equal to number of unique patrilines identified from the combined samples ($N = 36$ workers for each colony).

RESULTS

We obtained 300 mated queens from a total of 39 source colonies. Queens that came from the same source colony belong to the same female lineage (matriline). Ten queens (from eight different matrilines) died before being shipped to Houston; 109 queens (from 33 lineages) produced only eggs; six (from five lineages) produced larvae but no pupae, eight (from six lineages) produced only males; and 24 (from 13 lineages) produced fewer than 10 workers before the queen died. Six of the queens were the result of simultaneous ('batch') mating rather than sequential mating and were not included in most analyses. All queens from four of the 39 matrilines either died before producing offspring or produced fewer than 10 workers, leaving a total of 35 matrilines in the analyses represented by 137 queens.

The number of mates observed in the combined samples of worker offspring ($N = 36$ per colony) ranged from one to eight (Fig. 1). For 75% of the colonies, the two individual samples gave the same estimate of mating frequency, but in 20% of the colonies, the pooled sample increased the estimated mating frequency by one mate, and in the remainder of colonies it increased by two mates. On average, females mated with a mean ± SD of 3.79 ± 1.65 males ($N = 137$). Although, the sample size was small ($N = 6$), queens that were batch-mated showed a similar pattern (3–4 mates from 2 to 4 different male lineages). Substantial mating occurred during the first opportunity: approximately half of all matings were with the first pairing of male and female lineages (Fig. 2a). Overall, females were most likely to mate with the first males they encountered (66% of all females mated at least once with the first males they encountered) and were progressively less likely to mate with subsequent males (Fig. 2b).

Female lineages differed in measures of their likelihood of mating. Female lineages differed in mating frequency (ANOVA: $F_{34, 104} = 1.705$, $P = 0.021$; Fig. 3), although the differences were not dramatic. They also differed in the number of different male lineages with which they mated (ANOVA: $F_{34, 104} = 1.781$, $P = 0.014$), as well as in their propensity to mate with the first males they encountered (arcsine transformed proportions, ANOVA: $F_{34, 104} = 2.373$, $P = 0.0006$; Fig. 4).

The total number of mates was not correlated with the number of times a female mated with the first group of males (Pearson product-moment correlation: $r_{137} = 0.018$, $P = 0.84$). Females that mated at high levels initially tended to mate less with subsequent groups of males. Conversely, females that did not mate initially mated at higher levels with males in the second, third or fourth pairing (first versus later matings: $r_{137} = -0.587$, $P < 0.001$). The total number of mates was positively correlated with the total number of male lineages that a female mated with ($r_{137} = 0.425$, $P < 0.001$), indicating that most females did not mate to satiation with the first group of males they encountered. Average matriline mating frequency was uncorrelated with the average dry mass of gynes belonging to that matriline ($r_{33} = 0.006$, $P > 0.97$).

Consistent patterns of male lineage success across all mating opportunities would indicate that mating success is due to male lineage identity. Male lineages did differ in residual mating success (relative success in matings subsequent to the first), although the magnitude of the effect differed between days of the experiment (Fig. 5). One male lineage failed to mate successfully with any females from the matrilines to which it was exposed. Overall, the effect of male lineage identity on later mating success was highly significant (pooled $G_5 = 36.88$, $P < 0.001$). However, the mating success of a male lineage with its first group of females was not correlated with its success at mating with the second group ($r_{23} = 0.109$, $P > 0.63$; Fig. 6).

DISCUSSION

Female Mating Behaviour

We found modest but significant variation in mating frequency among female lineages (matrilines), suggesting that some of the natural variation in mating frequency we have observed in *P. occidentalis* may be a consequence of intrinsic variation among matrilines. Gynes from a particular matriline share a common rearing environment as well as a mother, but may be full or half sisters (because of multiple mating). Whether differences between

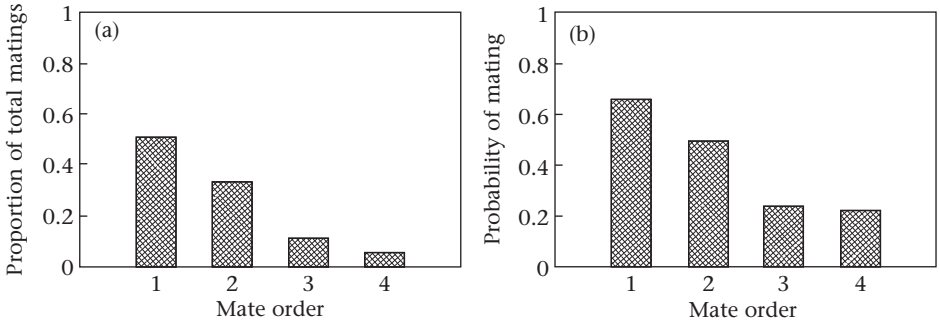

Figure 2. (a) Proportion of total matings obtained by male lineages to mate first, second, third or fourth with a female lineage. (b) Probability that a female mated at least once with the first, second, third or fourth group of males that she encountered.

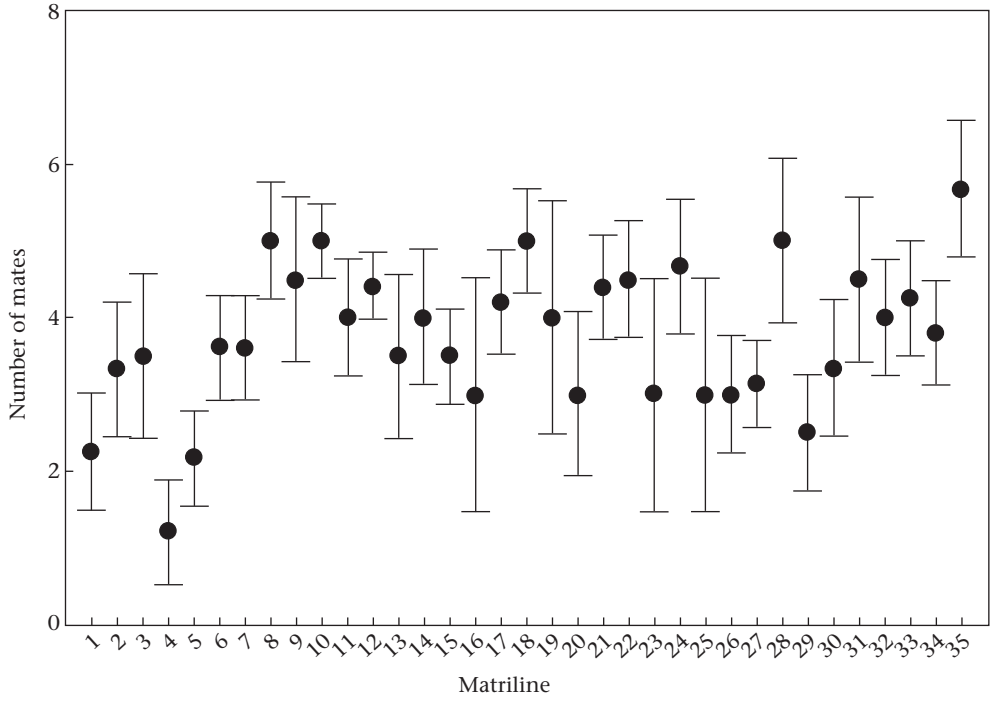

Figure 3. Variation among matrilines in mating frequency. Values are means ± SE mating frequency for females in each lineage.

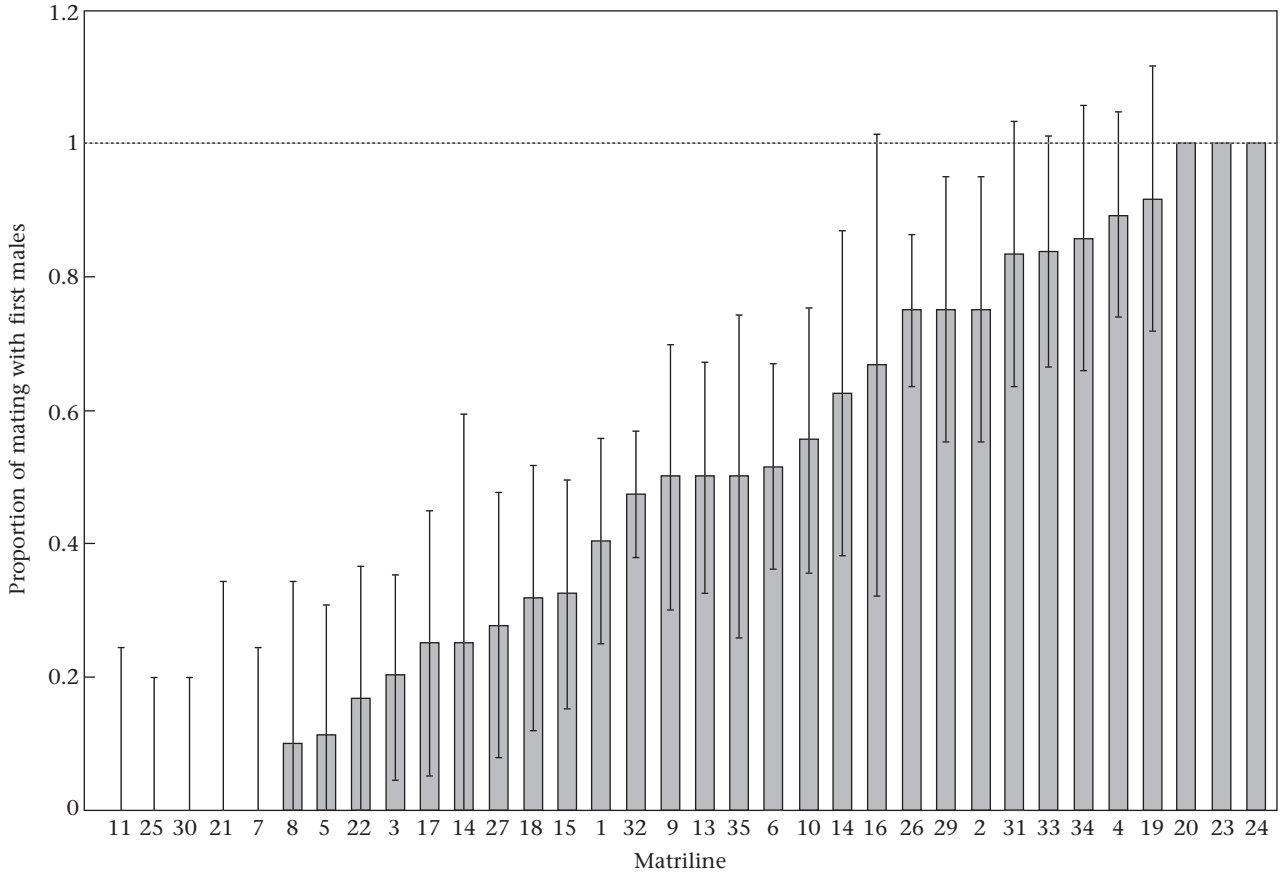

Figure 4. Variation among matrilines in the proportion of matings with the first male lineage they encountered. Bars represent mean ± SE proportions for females in each lineage.

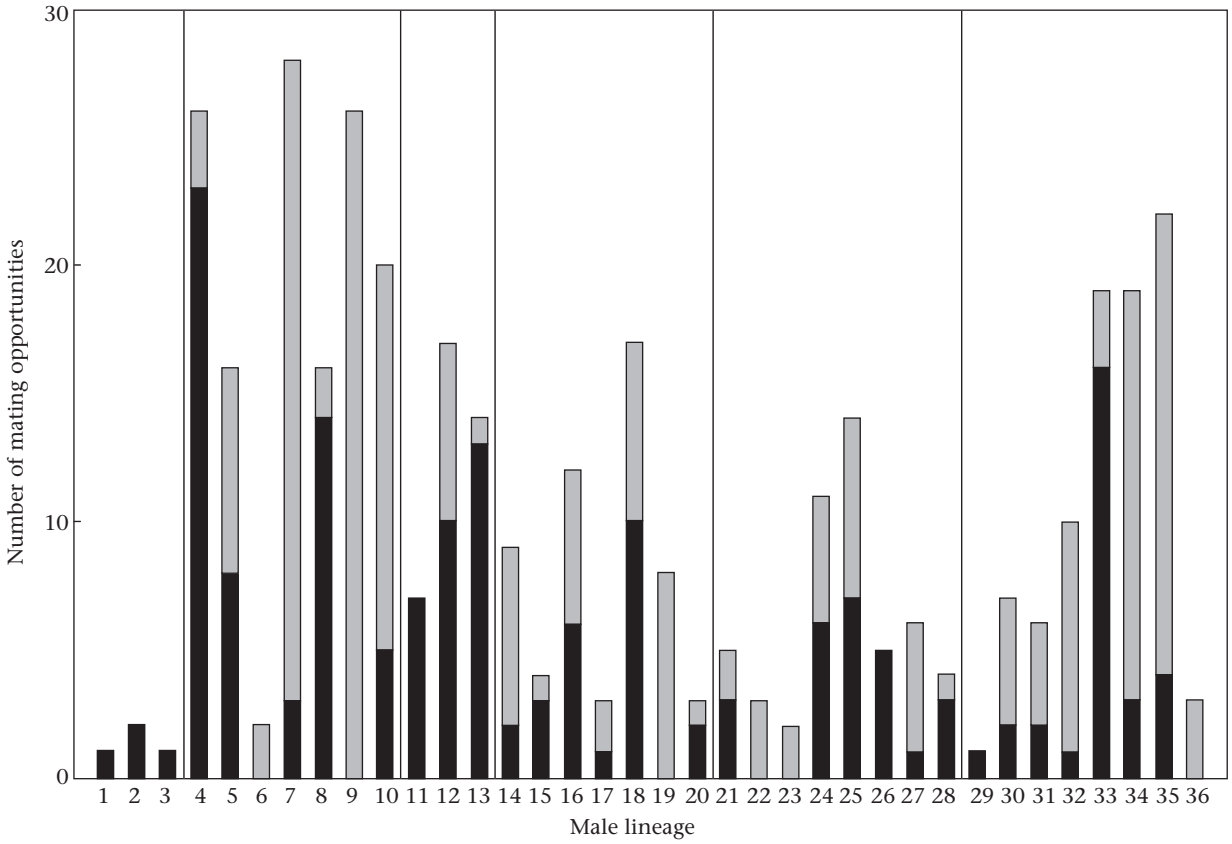

Figure 5. Variation among male lineages in residual mating frequency (all mating opportunities after the first). Vertical lines separate different days of the experiment. Black shading represents successful mating opportunities; grey shading represents unsuccessful ones.

matrilines represent maternal effects or genetic differences remains to be resolved. However, some previous studies in other species of insects have found little additive variance for mating behaviour but strong maternal effects (e.g. Shuker et al., 2007; Simmons, 2003). Size was not correlated with matriline mating frequency, suggesting that female size alone does not drive mating frequency, in contrast to some insect species (Bergström et al., 2002; McNamara et al., 2008). Although matrilines differ in the average size of gynes produced, among-colony variation is relatively slight due to the strong selective constraints on gyne size (Wiernasz & Cole, 2003).

The proportion of matings that occurred with the first male lineage to which the females were exposed varied dramatically among matrilines, again indicating that female lineages may vary intrinsically in their mating behaviour, as has been observed in other species (e.g. Wedell et al., 2002). These differences could result from variation in female mating propensity (e.g. Casares et al., 1998; Castrezana & Markow, 2008) or discrimination (Tregenza, Pritchard, & Butlin, 2000). The genetic diversity of males that females encountered under these experimental conditions would be much lower than at the mating swarm, possibly leading to more resistance from females. Differences among female lineages in readiness to mate (propensity) are unlikely to account for all of the variation because the initial differences in matriline mating frequency declined as females had more opportunities to mate. It is possible that females from some lineages were reluctant to mate at the start of the experiment but became more receptive as they were exposed to courting males and their pheromones. Alternatively, some matrilines may be more strongly discriminating than others (Pyle & Gromko, 1979).

If variation in mating frequency is largely a consequence of female mating behaviour, it suggests that some matrilines may have higher lifetime fitness primarily through the success of their daughters. Females that mated more also mated with males from more lineages, leading to greater genetic variation among the colony's workers. In *P. occidentalis*, colonies of queens that have mated with a large number of males grow faster than colonies of queens that have mated with few males, which in turn leads to higher survival, earlier reproductive maturity and greater reproductive success (Cole & Wiernasz, 1999; Wiernasz et al., 2004).

The number of matings we obtained was about half the number typically measured in wild collected females (Wiernasz et al., 2004). Given the benefits of multiple mating in this species, it is unlikely that females in natural populations are compelled to mate more than the optimal frequency. At our study site, mating swarms last 2.5–3 h, usually dissipating by 1800 hours (Abell et al., 1999). We do not know how much time an individual female spends in a reproductive swarm, but it is likely to be less than the entire time that the swarm is present (e.g. Reichardt & Wheeler, 1996). Our own observations at mating swarms suggest that females are continuously engaged with males. Although each female spent at least 1 h in contact with males, the disruption of being transferred between groups of males may have led to a delay in the onset of mating behaviour each time, reducing the overall mating frequency.

Male Mating Behaviour

Variation in male mating success may have been a function of differences among male lineages, an interpretation supported by the significant variation in residual mating success. Genetic

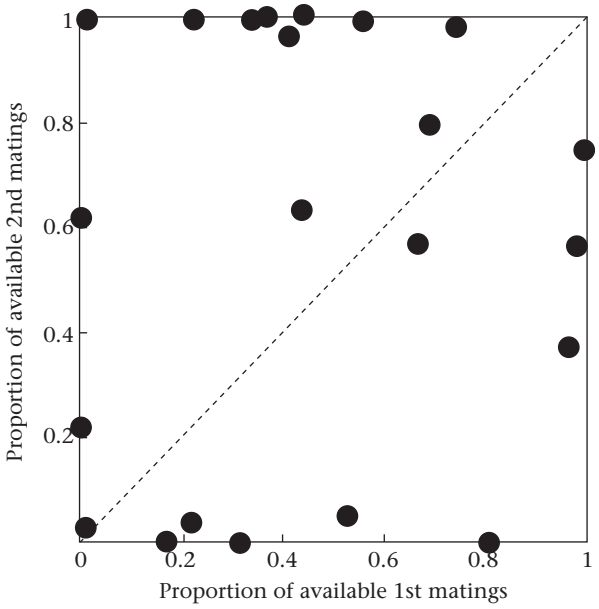

Figure 6. Correlation between mating success during the first and second opportunity for all male lineages with complete data. Dotted line is the line of equality.

variation in male mating success is characteristic of *Drosophila* (Hughes & Lieps, 2006) and has been found in other insect species (Solensky & Oberhauser, 2004; Wedell & Tregenza, 1999), and may contribute to the differences between male lineages. Variation in male courtship vigour may account for some of the male lineage failure; our observations during the experiment are consistent with some lineages courting more energetically than others. Some males may have mated successfully but transferred relatively few sperm, so that their genotypes were not among the sampled workers. The initial distribution of sperm in the spermatheca is probably highly heterogeneous (e.g. Wheldon, 1963), but is expected to become more homogeneous with time (e.g. Franck et al., 2002), especially in species that benefit from having a diverse worker force. We used two worker samples separated by 8 months because in reproductively mature *P. occidentalis* colonies, male genotype distributions among workers can vary significantly over time (Wiernasz & Cole, 2010). Although we cannot eliminate the possibility of 'missing' paternal lineages, we think that this is unlikely.

Interactions between Male and Female Lineages

Our ability to make inferences about male–female interactions is limited, but some interesting patterns emerge from our results. Although a few male lineages did consistently well and others poorly, overall, there was no relationship between mating success at the first and second opportunity. Despite this lack of correlation, there was significant variation among male lineages in residual mating success (realized mating opportunities with females after the first pairing), indicating that some lineages were highly successful in later mating (third and fourth pairing). These results suggest that male mating success depends upon which female lineages the males were paired with in the experiment. Variation in first male success rates may also reflect interactions between the sexes, with some initial combinations failing because of female resistance, perhaps on the basis of cuticular hydrocarbon profile (Howard & Blomquist, 2005). The lack of a positive correlation between a male lineage's success at first mating and its success at second mating supports this interpretation.

Another possibility is that some males mated but were incompatible with the female, leading to fertilization failure or to fertilized eggs that failed to complete development (Zeh & Zeh, 1996, 1997). Clark, Begun, and Prout (1999) documented strong interaction effects on fertilization among *Drosophila* lineages that had been selected for increased male offence or defence. Male–female genotype interaction has been observed for single matings between lineages of two ant species (Libbrecht et al., 2011; Schwander & Keller, 2008), although most of the effect is on the development of reproductive caste (gynes versus workers). However, studies that use single mating, especially in species where females typically mate multiply, cannot address the effects of other ejaculates on a male's mating success. The extent to which the pattern we observed are a consequence of matriline effects, paternal lineage effects, or more complex interactions will require further work.

Like those of its congener, *Pogonomyrmex rugosus* (Schwander & Keller, 2008), females of *P. occidentalis* mated readily when placed in a container with sufficient males. Their behaviour supports our assumption that females that were collected outside the colonies were reproductively competent. Our method was successful in generating multiply mated females and may apply to both *Pogonomyrmex* and other swarm-mating species for studying both mating and manipulating mating frequency in species where artificial insemination is not possible.

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