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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