

No evidence of pre-copulatory mate choice by gynes in the facultatively parthenogenetic ant *Cataglyphis cursor*

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ABSTRACT

Virgin queens (gynes) of the ant *Cataglyphis cursor* mate on the ground at the nest entrance, where they benefit from the protection of nestmate workers. By carrying out mating tests in the laboratory, we show that gynes mate with the first males they encounter, suggesting the absence of pre-copulatory mate choice by gynes. This is in agreement with the recent finding that workers behave aggressively towards foreign males and could thereby exercise a form of inclusive mate choice, which could replace pre-copulatory mate choice by gynes.

KEYWORDS

Eusocial insects, Sexual selection, Inclusive mate choice, Multiple mating.

Sexual selection favours phenotypic traits affecting reproductive success, for instance through better access to a sexual partner resulting from increased competitiveness among individuals of the same sex (intra-sexual selection) or from increased attractiveness towards individuals of the opposite sex (inter-sexual selection). While sexual selection has received considerable attention in solitary organisms it has been the subject of less attention in social species, especially in social insects (Davidson 1982; Boomsma *et al.* 2005; Boomsma 2007; den Boer *et al.* 2010; Boomsma 2013). This might be linked to the fact that in the latter sexual partners are committed for life, which minimizes the potential for sexual selection (Boomsma 2013). Furthermore, the widespread occurrence of nuptial flights associated with a high mortality of sexuals favours the evolution of traits that optimize access to partners (e.g. flight abilities, partner detection) but not those linked with pre-copulatory sexual selection (e.g. weaponry, ornaments, courtship behaviours) (Boomsma *et al.* 2005; Baer 2011).

However, some eusocial insects mate multiply on the ground near the nest entrance. This lowers the mortality of gynes (young virgin queens) since they are in the presence of protective workers and can retreat into the nest. Moreover, workers may directly participate in gyne mate choice, i.e. exercise a form of inclusive mate choice (Cronin *et al.* 2011). Indeed, gynes and workers have the same interests in choosing the “best” males. This is because workers typically do not reproduce and obtain indirect fitness by helping related queens. Workers would therefore benefit from helping related gynes to choose good mates,

and could achieve this by physically preventing males of poor quality from accessing gynes. Gynes would benefit from deferring mate choice to workers if doing so minimises their exposure to desiccation and predation. In addition, workers may collectively be in a better position to make an optimal choice than hard-pressed gynes.

The involvement of workers in mate choice has been suggested in army ants (Franks & Hölldobler 1987), Argentine ants (in the invasive range, Sunamura *et al.* 2011) and the facultatively parthenogenetic ant *Cataglyphis cursor* (Cronin *et al.* 2011; Helft *et al.* 2015). In the latter two species, workers are aggressive towards all foreign males who enter or come near the nest searching for a receptive gyne. Worker aggression may be a form of inclusive sexual selection by workers if they differentially affect some male phenotypes. This is indeed what occurs in *C. cursor*. Gynes are produced by the multiply mated queen (Lenoir *et al.* 1988; Pearcy *et al.* 2004) and workers are highly related to both parthenogenetically produced gynes (clones of their mother, $r \sim 0.5$) and sexually produced gynes ($r = 0.44$ on average) (Doums *et al.* 2013), and a recent study showed that worker aggression toward males affected male relative mating potential, increasing it for lighter males and decreasing it for heavier males (Helft *et al.* 2015). The present study therefore aims at determining whether *C. cursor* gynes exercise pre-copulatory mate choice or not. We observed mating behaviour of gynes in the presence of two males under laboratory conditions. The males differed in the level of harassment they had experienced from workers, with one of the two males previously exposed to worker aggression for 10 minutes while the other was not exposed to workers.

We used 115 sexuals (46 males and 69 females) from 22 colonies which had been produced in nature but which emerged in the laboratory, allowing us to be certain of their virginity (see Supplementary material). We carried out 23 mating tests, each using one focal

gyne and two males. In addition, two other gynes were used during the exposure phase of males to worker aggression immediately prior to the mating test (below). The focal gyne was placed alone in a mating arena to which it could habituate. Simultaneously, one male was placed in another box containing a second gyne in a mesh cage, so that he could perceive her presence but could not mate (treatment without workers or W-). A second male was placed in a third box containing the third gyne, also encaged, but with the addition of 50 of the gyne's nestmate workers that could aggress the male as shown in Helft *et al.* (2015) (male W+). After 10 minutes, the two males were moved to the mating arena containing the focal gyne and matings were observed for 10 minutes. All individuals were used only once. The three gynes used in each test were sisters and at least 19 days old, since previous observations suggest that most gynes are sexually active at this age. The two males were brothers and unrelated to the gynes. We selected males of the same age and size (see Supplementary material), hence they differed only in their previous exposure to worker harassment.

Mating attempts were unambiguous. The male mounted above the gyne with his front and middle legs embracing her thorax and only his hind legs remaining on the ground, made frenzied antennae contacts and attempted to copulate by rubbing the tip of his abdomen around that of the gyne. A successful attempt ended by the intromission of the male's genitalia into the female gaster for several seconds and was thus recorded as a mating even though we do not know whether sperm transfer occurred. Using BioLogic software (Dimitri Missoh, <http://swarmy.free.fr/wordpress/2009>) we recorded the latency before the first mating attempt, the number of mating attempts, and the number and duration of mating (i.e. duration of copulation). All statistical analyses were conducted using R-Gui software (version 3.0.2, 2008).

The gyne refused to mate with either male in nine out of the 23 tests. This may be because she was not receptive or stressed by the experimental setup, e.g. by the absence of nestmate workers or colony odour. Twenty matings occurred in the remaining 14 tests. They lasted $1 \text{ min } 10 \text{ s} \pm 30 \text{ s}$ (mean $\pm$ SD). *C. cursor* queens mate multiply (Lenoir *et al.* 1988, Pearcy *et al.* 2004) and multiple mating by gynes was indeed observed during our 10 minutes tests. Nine gynes mated once, four mated twice (two with the same male and two with both males) and one mated thrice (with both males). Similarly, males can mate multiply in this species (Cronin *et al.* 2011) and multiple mating by males was also recorded, with 14 males mating once and three mating twice (Fig. 1).

Although all W+ males were aggressed by workers during the exposure phase that immediately preceded the mating test (from 22 to 201 aggressions in 10 minutes, median = 94), this had no effect on the outcome of mating tests. W+ and W- males did not differ in latency before the first mating attempt (Wilcoxon test: $p = 0.9$), number of mating attempts (Wilcoxon test: $p = 0.7$), number of matings (Fisher exact test: $p = 0.54$) or duration of matings (Wilcoxon test: $p = 0.7$) (Fig. 1).

Both males attempted mating in 11 out of the 14 tests where mating occurred. The gyne always mated with the first male who attempted mating, usually on his second or third attempt, and she always mated before the second male attempted to mate (Fig. 1). This suggests that gynes mated indiscriminately, at least for their first mating, and supports the hypothesis that gynes largely defer pre-copulatory mate choice to workers (Helft *et al.* 2015). Note, however, that this does not preclude post-copulatory sperm selection (Baer 2011). For instance, cryptic mate choice by gynes could occur by rejecting the sperm of less fit partners and/or by controlling the duration of mating (Oppelt and Heinze 2007). In several instances we observed that gynes in copula turned around and bit the mounting

male and that this ended the copulation. This suggests that gynes have at least some degree of control over mating duration, and it is interesting in this context to note that mating duration was indeed very variable, lasting from 10 s to 1 min 57 s (Fig. 1).

The indiscriminate mating by gynes observed in our experiment is in apparent contradiction with previous field observations that gynes can refuse to mate and indeed actively avoid most mating attempts (Lenoir *et al.* 1988, Cronin *et al.* 2011). However, in these field observations gynes were likely already mated since most had lost their wings and wing loss is a good predictor of being mated (Doums *et al.* 2013), hence they were likely not seeking to mate but were seeking to be transported by workers to a new colony (Lenoir *et al.* 1988, Chéron *et al.* 2011). Further studies should investigate whether all matings result in the transfer of sperm and/or seminal secretions, whether mating duration affects this transfer, and whether gynes can adjust mating duration according to male quality or mating status.

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Figure 1: Timing of mating attempts and duration of matings. The x-axis shows the duration of the mating tests in min. On the y-axis, labels indicate colony identity, as well as gyne identity for colonies which provided multiple gynes. The shorter bars indicate mating attempts. A few that were largely overlapping have been slightly moved sideways for clarity. The taller bars indicate actual mating (copulation), with their width indicating the duration (given in s). W- males are in white and W+ males are in black.

