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

Supplementary material

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MATERIAL AND METHODS

The study species

Colonies of *Cataglyphis cursor* contain on average 700 workers and are headed by a single multiply mated queen (Lenoir *et al.* 1988; Pearcy *et al.* 2004). Males and gynes are produced in spring following a period of overwintering , with a skewed sex ratio in favour of males (4:1, Pearcy and Aron 2006). Mating generally takes place from mid-May to mid-June (Cronin *et al.* 2011) before new colonies are produced by fission (Lenoir *et al.* 1988, Chéron *et al.* 2011a). The queen mates with on average 5 to 6 foreign and unrelated males (Pearcy *et al.* 2009; Doums *et al.* 2013a). This species is unusual in that the queen produces workers through sexual reproduction and gynes through thelytokous reproduction (Pearcy *et al.*

2004) and/or sex (Doums *et al.* 2013b). Workers are also capable of using thelytoky to produce gynes, but this is mainly restricted to when colonies become orphaned (Cagniant 1979; Clémencet *et al.* 2008; Chéron *et al.* 2011b).

Collection and rearing

To obtain field-produced sexuals we collected 17 queenright colonies in a population of *C. cursor* at Argelès-sur-mer (Pyrénées Orientales, France) in early May 2012, i.e. after sexual cocoons were produced but before adults emerged. These colonies were housed in the laboratory, where eight colonies produced males, six produced gynes and three produced both males and gynes. In addition, to obtain field-produced gynes we had previously collected five additional colonies that we orphaned and transplanted into enclosures exposed to natural conditions (for details see Cronin *et al.* 2012) in late September 2011, prior to overwintering, in order to trigger gyne production by workers in spring 2012. These orphaned colonies were then re-collected in May 2012, as in Helft *et al.* (2015), that is after queen cocoons were produced but before adult emergence. These colonies were also housed in the laboratory, where gynes emerged.

Once in the laboratory (28°C, D:L 15:9), colonies were kept in plastic boxes (25 x 35 x 15 cm) with a plaster floor dug on one side to provide ants with a shelter covered with glass. Ants were fed three times a week with mealworms and meringue, and had sugar and water *ad libitum* at their disposal.

Males were collected from four to seven days after emergence, when they went out of the nest and ran excitedly around the foraging area, often with wings semi-opened. They were carefully weighed and marked (using Mitsubishi paint pens) with two colour dots on the thorax recording the date, so as to record their approximate age. They were then stored

with other sexually mature brothers kept in a plastic box of 25 x 35 x 20 cm (one box per colony) until they were used in mating tests.

Gynes were collected two days after their emergence, i.e. once they had completed cuticle melanisation, and colour marked on the thorax. They were not returned to their colony because of the risk that they would be killed as *C. cursor* colonies rapidly restore monogyny (Clémencet *et al.* 2008; Chéron *et al.* 2011a). Instead, each gyne was kept with a group of nestmate workers (approximately one third foragers and two thirds nurses) separately from her colony until being used.

Experimental design

We used a paired design with 23 replicates, using a total of 46 males from 11 colonies and 69 gynes from 14 colonies. All individuals were used only once in the experiment. However, 14 replicates were not truly independent as although they used different individuals these came from the same colonies. Specifically, the same pair of male-producing and gyne-producing colonies was used for five replicates, and two other colony pairs were used for three replicates each. In addition, three male-producing colonies were used twice each (paired with six different gyne-producing colonies). Using a single replicate for each colony did not qualitatively change the results, thus we considered all replicates as independent in the statistical analysis.

Since male age and size may influence mating success (Amin *et al.* 2012) we controlled for these factors by using two brothers of the same age and of the same size for each replicate. We selected brother males that were highly active in their isolation box (running, trying to fly), approximately of the same age (same colour marks) and of similar size (checked visually first, and then by weighing). Therefore, within each replicate the two

males only differed in their exposure to worker aggression. Each replicate also used three sister gynes from another colony (Fig. S1).

Exposure phase

Two arenas of 25 x 35 x 20 cm with a plaster floor were set up for the exposure phase as follows. We carefully took one gyne that had been isolated with a group of 50 workers, for two weeks or more, and put her in a small mesh cage. We then placed the nest containing the 50 workers in one of the arenas, and placed the mesh cage with the gyne on top of the nest. The arena thus contained 50 workers with their nest and one gyne (treatment W+). Placing the gyne in a mesh cage made her perceptible to and accessible by males, yet it prevented mating so that it would be possible to test the effect of worker harassment on male mating in the following phase of the experiment (Fig. S1). We proceeded similarly with the other arena except that we did not add workers. This arena thus contained a nest that had been recently occupied by 50 workers, and a gyne in a mesh cage (treatment W-).

The ants were left to settle for 20 minutes, to allow gynes to calm down and workers to explore the arena. One male was then introduced into each arena. We videotaped the exposure phase to measure male activity in both treatments, and interactions with workers in treatment W+. As expected, treatment W+ resulted in numerous aggressions of the male by workers (from 22 to 201 aggressions in 10 minutes, median = 94). At the end of the 10 minutes of the exposure phase, the males of each treatment were carefully moved to the mating arena with the focal gyne (plastic box with a plaster floor 20x20x20 cm) and matings were recorded and quantified.

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Figure S1: Experimental design. During the exposure phase (upper section), two males were each put in an arena where a gyne was encaged. This arena either contained no worker (W-) or contained 50 workers related to the gyne (W+). Immediately upon completion of the exposure phase, the two males were transferred in another box containing the focal gyne (lower section). This gyne was not encaged hence mating could occur. Each phase lasted 10 minutes.