



Inbreeding effects on worker size in the ant *Cardiocondyla elegans* (Hymenoptera: Formicidae)

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Abstract. Although outbreeding is common among ants, genetic data suggest the regular occurrence of sib-mating in several species of the genus *Cardiocondyla*. Here, we demonstrate that workers produced by queens of *Cardiocondyla elegans* that mated with unrelated males are significantly larger than those produced by queens that mated with their brothers. Although this difference was small, prolonged inbreeding could have negative fitness consequences and may thus explain our previous observation that workers carry their sexual sisters to foreign nests, hereby promoting outbreeding.

INTRODUCTION

Inbreeding is often detrimental to the fitness of a species. This is particularly the case in haplodiploid social insects with complementary sex determination, where mating between siblings can result in a high proportion of fertilised eggs developing into sterile, diploid males instead of diploid workers or female sexuals (Harpur et al., 2013; Leung & van der Meulen, 2022; Gokhman et al., 2026). However, a few ant genera have evolved alternative mechanisms of sex determination, which avoid the production of diploid males. One such genus is *Cardiocondyla*, which is characterised by the occurrence of non-dispersing, wingless males that mate with young queens in their natal nests. Estimates of inbreeding coefficients from genetic markers suggest that over 80% of all matings involve first-degree relatives (Schrempf et al., 2005; Heinze et al., 2014; Vidal et al., 2021). Even after ten generations of brother-sister mating or one episode of mother-son mating in the laboratory, no diploid males were produced (Schrempf et al., 2006; Schmidt et al., 2014). Nevertheless, prolonged sib-mating appears to result in a shorter queen life span, higher brood mortality, and a more male-biased sex ratio (Schrempf et al., 2006).

Several tropical species of *Cardiocondyla* mitigate these negative effects by occasional outbreeding involving winged, dispersing males or by adopting alien queens into facultatively multi-queen colonies (Heinze, 2017). How-

ever, these strategies are not applicable to species from temperate, xeric areas in Central Asia or the Mediterranean region, where winged males have been completely lost and obligate single-queening has evolved (Schrempf & Heinze, 2007). At least one species, *C. elegans*, employs a fascinating strategy to promote outbreeding: its workers transport female sexual relatives to other nests for mating with multiple unrelated males (Vidal et al., 2021; Vidal & Heinze, 2022). During “matchmaking,” the tiny 2 mm-long workers cover distances of up to 16 m at soil temperatures of around 35°C and are occasionally met with aggression at the nest entrance of the recipient colony. This indicates that the strategy is costly, raising the question of the immediate benefits of outbreeding. A previous study (Vidal & Heinze, 2022) found that, during the first seven weeks after hibernation, queens that mated with their siblings produced a similar number of offspring to queens that mated with one or more unrelated males. Here we explore whether inbred and outbred offspring workers differ in morphology or show signs of developmental stress.

MATERIAL AND METHODS

In late August 2019, workers carrying female sexuals were collected near Remoulins on Gardon (43°55'43.9"N, 4°34'5.1"E) and transferred to the laboratory. There, 155 individual queens were kept in isolation (n = 112) or with one (n = 16), two (n = 13) or three (n = 14) unrelated males for one day. The males were then

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removed and the female sexuals were placed individually into 94 mm × 16 mm Petri dishes (see Vidal & Heinze, 2022 for details) and kept in incubators under near-natural conditions (17°C night / 34°C day from June to September, slowly decreasing to 4–5°C night / 13°C day from December to March). The queens were fed with honey, dead *Drosophila* and chopped-up cockroaches twice per week. Only a small percentage of the queens produced sufficient numbers of offspring before dying (without males: 7 out of 112 queens; with one male: 2 out of 16 queens; with two males: 6 out of 13 queens; with three males: 3 out of 14 queens). The mating frequency of these 18 queens was determined by genotyping ten worker offspring per queen at seven microsatellite loci (see Vidal & Heinze, 2022). According to these genotypes all queens had mated with one to seven unrelated males and their offspring were therefore considered to be outbred.

We compared the morphology of the outbred offspring workers of these 18 queens with that of the inbred offspring of 22 queens which had not had the opportunity to mate with unrelated males, meaning that they had only mated with their brothers. These queens and their mates emerged in summer 2019 from four stock colonies that were collected at the same site in 2018. The stock colonies were kept in the laboratory under the aforementioned conditions. After isolation, sib-mated queens were kept under the same conditions as the field-collected queens.

We measured the following morphological traits of three mounted offspring workers per queen using a Wild M 10 stereomicroscope equipped with a 1.6× plan-apochromatic objective at 40–63× magnification: head width including the eyes, head length, mesosoma length, and the lengths of the left and right antennal scapes and prothoracic tibiae. As these values depend on the exact orientation of the specimen, we determined the approximate measurement error by measuring the left scape twice as an example. The average difference between the two values was 1.315%. We investigated the impact of the following independent factors: inbreeding vs. outbreeding; the number of alien mates (one vs. multiple as determined by genotyping at seven microsatellite loci, see Vidal et al., 2021; Vidal & Heinze, 2022); and queens that mated in the field vs. queens that mated in the laboratory. We checked the data for normality before conducting a main-effects ANOVA using Statistica 6.0 (StatSoft, 2001).

We estimated fluctuating asymmetry crudely by comparing the differences in the left and right tibia and antennae of the various queen categories. Due to the negative results of this initial analysis, we decided against conducting a more comprehensive, time-intensive one.

RESULTS AND DISCUSSION

The main effects ANOVA revealed that neither the location in which queens mated (laboratory vs. field) nor the number of matings (single vs. multiple) had a significant impact on the morphometry of offspring workers ($p > 0.05$). Inbreeding appeared to lead to narrower heads, both in absolute terms (inbred, $n = 66$: $499 \pm \text{SD } 20 \mu\text{m}$, outbred, $n = 54$: $525 \pm 19 \mu\text{m}$; $F = 17.50$, $p < 0.0001$; Fig. 1) and relative to head length ($F = 10.67$, $p = 0.0014$), as well as a shorter mesosoma (inbred: $628 \pm 26 \mu\text{m}$, outbred: $660 \pm 27 \mu\text{m}$; $F = 6.73$, $p = 0.0107$). After Benjamini-Hochberg correction for multiple tests, the effect of inbreeding on both absolute and relative head width remained significant.

Although the absolute lengths of the scapes and tibiae were unaffected by any of the independent variables, inbreeding appeared to have a slight, albeit non-significant,

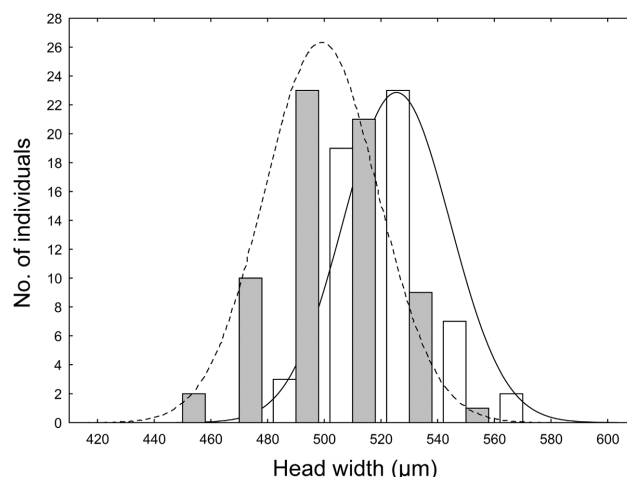


Fig. 1. Frequency distribution of the head width of workers of the ant *Cardiocondyla elegans* produced either by queens that mated with their brothers (hatched bars) or by queens that mated with alien males (open bars).

effect on the fluctuating asymmetry of the scapes ($F = 3.56$, $p = 0.0618$). When the significant tests were repeated using only the mean of three offspring workers per queen to avoid pseudoreplication, the results concerning the impact of inbreeding on absolute head width ($F = 13.09$, $p = 0.0009$), relative head width ($F = 8.00$, $p = 0.0076$), and mesosoma length ($F = 4.38$, $p = 0.0435$) were corroborated.

In Hymenoptera, sib-mating often results in the production of large numbers of diploid males instead of workers (Harpur et al., 2013; Leung & van der Meulen, 2022; Gokhman et al., 2026). In contrast, *C. elegans* queens that were given the opportunity to mate only with their brothers regularly produced female offspring, with no evidence of diploid males. However, the morphology of the offspring of sib-mated queens differed from that of the offspring of queens that had mated with one or several alien males. Outbred workers had on average wider heads and a longer mesosoma, even though the difference was only about 5%. Similar influences of inbreeding on body size have been observed in several other insects, including field crickets *Gryllus campestris* (Tregenza et al., 2026) and butterflies (Pahnke, 2009).

Although body size is often associated with fecundity and other fitness traits, such as the dependence of foraging on temperature (Wendt & Verble-Pearson, 2016), the consequences of the observed differences in *C. elegans* remain unclear without further research. First, as *Cardiocondyla* workers lack ovaries and are therefore unable to produce offspring, changes in body size cannot affect their direct fitness. Second, the 5% difference is probably too small to have an immediate biological impact on traits such as foraging or brood care efficiency. However, prolonged inbreeding over many generations may amplify subtle effects, resulting in changes to life expectancy, sex ratio, and queen fecundity, as has been observed in *C. obscurior* (Schrempf et al., 2006). Promoting outbreeding by transferring female sexuals between nests may therefore be an important strategy for maintaining population fitness (Vidal et al., 2021).

One caveat should be noted: all inbred offspring originated from queens that emerged in the laboratory, whereas outbred offspring came from queens that emerged in the field, although they mated in the lab. Although there is no evidence from other studies to suggest that lab-reared queens produce smaller workers and field- and lab-reared queens did not differ in fecundity (Vidal & Heinze, 2022), the results of our study should be considered preliminary with further rearing experiments required.

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CONFLICTS OF INTEREST. The authors declare that they have no conflict of interest.

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