

Research article

Production of winged and wingless males in the ant, *Cardiocondyla minutior*

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Summary. Wingless (ergatoid) males of the tramp ant *Cardiocondyla minutior* attack and kill their young ergatoid rivals and thus attempt to monopolize mating with female sexuals reared in the colony. Because of the different strength of local mate competition in colonies with one or several reproductive queens, we expected the production of new ergatoid males to vary with queen number. Sex ratios were mostly female-biased, but in contrast to the sympatric species *C. obscurior* (Cremer and Heinze, 2002) neither the percentage of ergatoid males nor of female sexuals among the first 20 sexuals produced varied considerably with queen number. As in *C. obscurior*, experimental colony fragmentation led to the production of winged males, whereas in unfragmented control colonies only ergatoid males eclosed.

Key words: Male polymorphism, local mate competition, ergatoid male, sex allocation; *Cardiocondyla*.

Introduction

In animals with random mating, parents typically invest equally in their male and female offspring and population sex ratios are close to 1:1 (Fisher, 1930). This may be strikingly different in species in which males do not disperse but locally compete with their relatives for access to the females (local mate competition, Hamilton, 1967). In such cases, mothers benefit most from producing a highly female-biased sex ratio instead of rearing numerous sons, which only would reduce each other's mating success.

In ants and other social insects, female-biased sex ratios are typically explained as worker control of sex allocation (Trivers and Hare, 1976; but see Alexander and Sherman, 1977). Local mate competition appears to be of restricted importance in ants, because in most species mating occurs during large nuptial swarms, where male and female sexuals from dozens or hundreds of nests meet (Hölldobler and Bartz, 1985). A striking exception is the tiny myrmicine *Car-*

diocondyla obscurior (Wheeler, 1929) (before 2002 referred to as *C. wroughtonii* Forel, 1890; Seifert, 2003). Males of *C. obscurior* are either wingless and workerlike in appearance, with strong, sickle-shaped mandibles ("ergatoid males"), or winged and resembling the ordinary males of related genera. Ergatoid males attack other ergatoids, grasp and bite them with their mandibles, and apply droplets of a whitish fluid from the gaster tip to their opponents' bodies (Kinomura and Yamauchi, 1987; Stuart et al., 1987; Yamauchi and Kawase, 1992). This secretion apparently elicits strong worker aggression, resulting in the elimination of the besmeared male (Yamauchi and Kawase, 1992). Colonies therefore typically contain only a single ergatoid male, which may monopolize mating with all female sexuals eclosing in the colony over a period of several weeks (Kinomura and Yamauchi, 1987). Winged males, in contrast, are docile and not well equipped for fighting. During the first days of their adult life, they also engage in sexual activities in the nest, avoiding attacks by ergatoid males through mimicking virgin queens, but after approximately one week they emigrate to mate with queens outside (Cremer et al., 2002).

Due to local mate competition, *C. obscurior* colonies with one mother queen produce only few ergatoid males, while the production of ergatoid males is increased with rising numbers of reproductive queens per nest. The percentage of winged males in a colony's sexual offspring appears to be unaffected by the number of mothers (Cremer and Heinze, 2002). In contrast, the production of winged males likely depends on environmental conditions (Cremer and Heinze, 2003).

Ergatoid males have as yet been found in all *Cardiocondyla* species, from which males are known (Kugler, 1983; Heinze, 1999; Seifert, 2003). However, ergatoid males of *C. obscurior* and a few related species (e.g., *C. wroughtonii*, Seifert, 2003) are atypical in that they have elongated mandibles and regularly attack also adult rivals. In other species, such as *C. mauritanica*, *C. emeryi*, *C. kagutsuchi*, and *C. minutior*, fighting appears to be less common among adult males, and ergatoid males use their strong mandibles predominantly to crush the soft cuticle of freshly eclosed

ergatoids (Heinze et al., 1993, 1998; but see Yamauchi and Kinomura, 1993 for regular adult male fighting in *C. minutior* from Okinawa). Because some callow males occasionally remain undetected, several adult males may co-occur especially in large colonies of these species (Heinze et al., 1998; Anderson et al., 2003). Furthermore, *C. obscurior* and *C. wroughtonii* nest in ephemeral cavities in plant material, such as coiled leaf of lemon trees or aborted coconuts, whereas colonies of the other species inhabit holes in the ground.

Aim of our present study was to investigate whether the pattern of adaptive regulation of sex allocation in response to varying queen number previously reported for *C. obscurior* (Cremer and Heinze, 2002) holds also for other facultatively polygynous *Cardiocondyla* species with less aggressive ergatoid males and different nest architecture. Using *C. minutior* as a sympatric model system, we also examined whether colony fragmentation induces the production of winged males, as it does in *C. obscurior* (Cremer and Heinze, 2003).

Methods

Cardiocondyla minutior is a widely distributed tramp species in the Pacific islands and the neotropics (e.g., Harris and Berry, 2001). Originally described from Hawaii as a variety of *C. nuda* (Forel, 1899), it was later synonymized with *C. nuda* (Wilson and Taylor, 1967) but is now recognized as a separate species (Heinze, 1997; Seifert, 2003).

Colonies with approximately 30 to 50 workers and several queens each were excavated from their nests in the soil in August 2000 at Arno's Vale Bay, Plymouth (Trinidad and Tobago) and in September 2000 at CEPLAC near Itabuna (Bahia, Brazil). We might have failed to collect the colonies completely because their nests consist of numerous small chambers in the ground. In the laboratory, these "stock colonies" were housed in small plastic boxes with a plaster floor as previously described (Heinze et al., 1998). They quickly grew to large size, with up to several hundred workers.

To allow a direct comparison with *C. obscurior* we exactly followed the experimental procedure described by Cremer and Heinze (2002). From ten large stock colonies (six from Tobago and four from Bahia) we set up experimental subcolonies with a standardized number of 50 workers and one, two, five, or ten queens each (ten colonies per queen number). It was not always possible to establish all four subcolonies from a single stock colony. Furthermore, in some cases, two or more replicates originated from the same stock colony. However, as most populations of tramp species are to some degree unicolonial, the sampling of many more stock colonies would not have protected against the risk of lacking independence of data and pseudoreplication.

To determine whether the number of reproducing queens had an effect on the production of the three types of sexual offspring (female sexuals, ergatoid males, winged males), we collected the first sexuals reared in each experimental subcolony immediately after their eclosion (to avoid interference with newly hatching sexuals). Because *Cardiocondyla* workers do not possess any ovaries (Heinze et al., 1993), all male and female sexuals were produced by queens. As in our previous study (Cremer and Heinze, 2002), we deliberately limited the number of sexuals investigated to 20, as subcolonies later differ strongly in size due to different production of workers and also because larger colonies are characterized by the production of almost exclusively female sexuals. Due to the death of some reproductive queens before the end of the experiment, three subcolonies had to be excluded from the analysis, four were analyzed with only 19 sexuals, and one with only 18 individuals. In other subcolonies, more than 20 (up to 28) sexuals were collected due to simultaneous eclosion of several sexuals in the offspring (median number of sexuals for all colonies: 22, quartiles 20 and 23).

Though *C. minutior* was presumably only recently introduced to South America and the two populations did not differ in any of more than 1000 sequenced base pairs of the mitochondrial COI/COII gene (A. Eberhardt, pers. comm.), it cannot be assumed a priori that the two populations are identical in their reaction to changes in queen number. We therefore analyzed the larger data set from Tobago separately. Because data were not normally distributed, numerical sex ratios and the percentage of ergatoid males among all sexuals produced were compared between subcolonies with different queen number using non-parametric tests.

Results

As observed in *C. obscurior* (Cremer and Heinze, 2003), the experimental fragmentation of colonies led to the production of winged males in 33 of our 37 subcolonies, while the stock colonies continued to rear exclusively ergatoid males, female sexuals, and workers during the whole course of the experiment. Subcolonies were significantly more likely to produce winged males than unmanipulated stock colonies (χ^2 -test, Yates corrected $\chi^2 = 28.79$, $p < 0.001$). Interestingly, all ten *C. minutior* stock colonies simultaneously produced large numbers of winged males several months after the end of our experiment, from September 2002 to January 2003, though they still contained hundreds of workers and rearing conditions were unchanged. Ergatoid males in stock colonies moved around on the brood pile and efficiently killed most freshly eclosing ergatoid rivals (see also Heinze et al., 1998), but some colonies nevertheless contained two or three ergatoid adults, suggesting that callow males occasionally escape or survive attacks.

The numerical sex ratio among the first 20 sexuals produced in the 37 subcolonies originating from stock colonies from Tobago and Bahia was female-biased (median 61.9% female sexuals, quartiles 39.5 and 81.3%, Fig. 1a) and did not differ among subcolonies with different queen numbers (median test, $\chi^2 = 2.486$, $df = 3$, $p = 0.478$). It also differed neither between monogynous and polygynous (Mann-Whitney U-test, $U = 115$, $p = 0.494$) nor between subcolonies with few (1, 2) or many (5, 10) queens ($U = 128$, $p = 0.191$). A separate analysis of the data set from Tobago did not change these results (median sex ratio: 61.9% female sexuals, quartiles 31.6 and 81.3%; median test: $\chi^2 = 3.444$, $p = 0.328$; monogynous vs. polygynous subcolonies: $U = 67$, $p = 0.868$; few vs. many queens: $U = 63$, $p = 0.200$).

Similarly, the percentage of ergatoid males among the first sexuals eclosing did not differ with queen number (range 4.1 to 45.0%, median 9.1%, quartiles 4.9 and 13.4%; median test, $\chi^2 = 2.161$, $p = 0.534$, Fig. 1b) and it also differed neither between monogynous and polygynous subcolonies ($U = 71$, $p = 0.343$) nor between subcolonies with few or many queens ($U = 76.5$, $p = 0.105$). Excluding the subcolonies from Bahia gave the same results (range, medians quartiles as above; median test: $\chi^2 = 3.4438$, $p = 0.328$; monogynous vs. polygynous subcolonies: $U = 52.5$, $p = 0.341$; few vs. many queens: $U = 58$, $p = 0.118$).

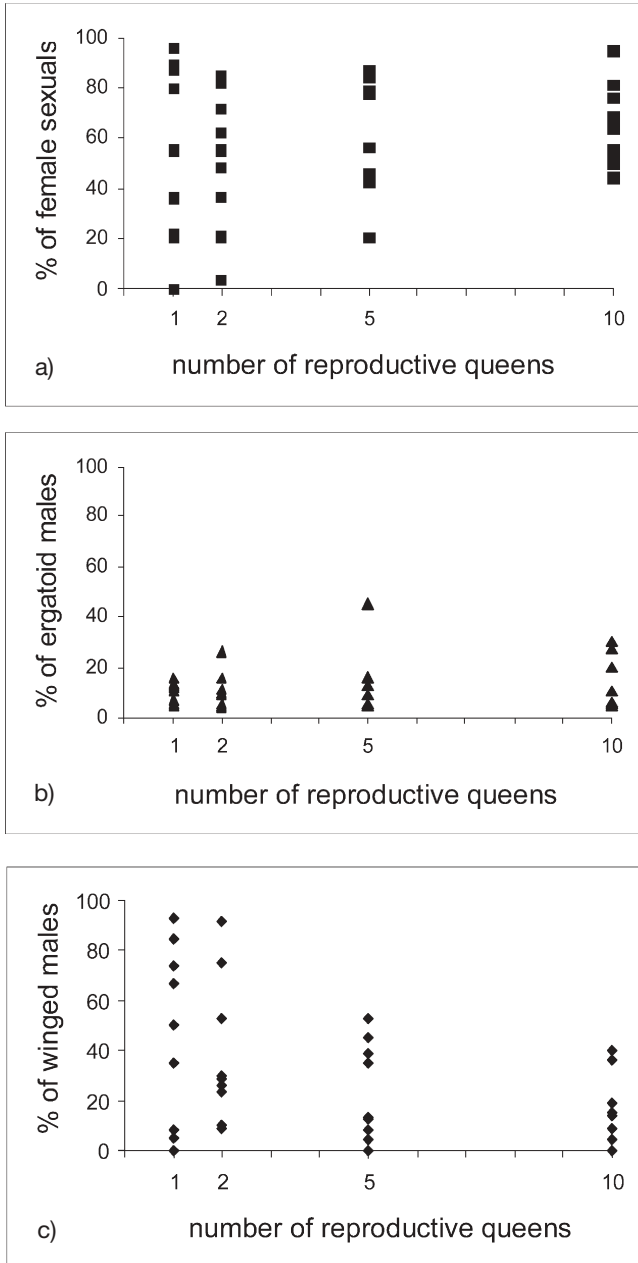


Figure 1. Scatter plot of percentages of a) female sexuals, b) ergatoid males, and c) winged males (on total sexuals) produced in subcolonies of *Cardiocondyla minutior* in relation to the number of reproductive queens (1, 2, 5, or 10) per subcolony

Discussion

Fragmentation of colonies of *C. minutior* induced the production of large numbers of winged males in the resulting subcolonies, as previously observed in *C. obscurior* (Cremer and Heinze, 2003), suggesting that a similar mechanism underlies phenotypic plasticity. This response to the decrease of worker numbers per nest appeared to be even more pronounced in *C. minutior* than in *C. obscurior*, in that both the

absolute number of winged males among the first 20 sexuals reared and the percentage of subcolonies producing winged males after manipulation were higher in *C. minutior* than in *C. obscurior*. Anecdotal evidence suggests that winged *C. minutior* males may also be spontaneously produced in unmanipulated colonies: all 10 stock colonies of *C. minutior* from Tobago and Bahia started to simultaneously produce winged males in fall 2002, a few months after the end of our experiment. Production of winged male sexuals in fall has also been observed in laboratory colonies of *C. minutior* from Okinawa (referred to as “hime-hadaka-ari” by Yamauchi and Kinomura, 1993; described as *C. tsukuyomi* by Terayama, 1999 and later synonymized with *C. minutior* by Seifert, 2003). As yet it is unclear what induced this sudden production of winged males, but an innate seasonality might serve to synchronize mating activities between different colonies and thus help to ensure regular outbreeding.

In contrast to the equal response to colony fragmentation, *C. minutior* differed from *C. obscurior* in its reaction to the degree of local mate competition in the nest (respectively queen number). In *C. obscurior*, an increasing number of reproducing queens in experimental colonies led to an increase in the production of ergatoid males and to a decrease of female sexuals (Cremer and Heinze, 2002). Using exactly the same experimental set-up as in *C. obscurior* and equally limiting the analysis to the first 20 produced sexuals, in *C. minutior* we could not detect any influence of queen number on sex ratio or the percentage of ergatoid males. The investment in ergatoid males was generally lower in *C. minutior* than in *C. obscurior*, even in single-queen subcolonies, in which *C. obscurior* produces a minimum number of ergatoid males. Our finding, that the production of ergatoid males is not or less strongly affected by queen number in *C. minutior* might suggest that the investment is generally so low that in monogynous subcolonies it cannot be further reduced without running the risk of not having enough local males for insemination of the newly hatching female sexuals in the colony.

Numerical sex ratios of *C. minutior* differed considerably between subcolonies but on average were female-biased, regardless of queen number. In agreement with our data from colonies from Bahia and Tobago, the percentage of female sexuals among all sexuals produced ranged between 47% and 86% in *C. minutior* colonies from Okinawa, Japan (Yamauchi and Kinomura, 1993).

At present it is unclear why *C. minutior* and *C. obscurior* differ in their production of ergatoid males and their response to varying queen number. The two species appear to have similar nest sizes (30 to 50 workers and 1 to 15 queens; Heinze, 1999 and S.C. unpublished data), though the extent of polydomy is not known. As mentioned above, the two species differ in nesting sites and male behavior. *C. obscurior* nests aboveground in small, fragile cavities in plants, while *C. minutior* lives in a network of small nest chambers in the soil. Such soil nests are probably more stable than the ephemeral plant nests and reduce the probability of changes in queen number and of variation in the degree of local mate

competition. Furthermore, due to differences in the fierceness of male fighting between the two species, additionally produced ergatoid males of *C. minutior* are not necessarily such a “wasted investment” as in *C. obscurior*. More data, in particular on nest site stability and the size of natural polydomous colonies, might help to clarify the cause for the observed differences.

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