Queen number influences the timing of the sexual production in colonies of Cardiocondyla ants

Masaki Suefuji, Sylvia Cremer, Jan Oettler and Jürgen Heinze*

*Author for correspondence (juergen.heinze@biologie.uni-regensburg.de).

Wingless males of the ant genus Cardiocondyla engage in fatal fighting for access to female sexual nestmates. Older, heavily sclerotized males are usually capable of eliminating all younger rivals, whose cuticle is still soft. In Cardiocondyla sp. A, this type of local mate competition (LMC) has turned the standard pattern of brood production of social insects upside down, in that mother queens in multi-queen colonies produce extremely long-lived sons very early in the life cycle of the colony. Here, we investigated the emergence pattern of sexuals in two species with LMC, in which males are much less long-lived. Queens of Cardiocondyla obscurior and Cardiocondyla minutior reared their first sons significantly earlier in multi-queen than in single-queen societies. In addition, first female sexuals also emerged earlier in multi-queen colonies, so that early males had mating opportunities. Hence, the timing of sexual production appears to be well predicted by evolutionary theory, in particular by local mate and queen-queen competition.

Keywords: local mate competition; fatal fighting; emergence order; Formicidae

1. INTRODUCTION

Species with local mate competition (LMC, Hamilton 1967) are valuable model systems for testing predictions from evolutionary theory. LMC occurs when sexuals do not disperse and locally compete for mating partners. In accordance with theory, mothers, who alone produce the offspring in a patch, rear just a few weeks (Heinze et al. 1999, 2005) and short-lived, wingless fighter males eclose long before female sexuals and occasionally even before the first workers. These early males eliminate later eclosing rivals and, owing to their extraordinarily long lifespan of up to one year, mate with all female sexuals that are later produced in the colony. By producing male offspring ‘ahead of time’, mother queens increase the chance that their own sons survive and reproduce (Yamauchi et al. 2006).

The reproductive tactics of Cardiocondyla males are very plastic in evolution (Heinze et al. 1999, 2005) and so is male longevity. In most species, males live for only a few weeks (Heinze et al. 1998; Schrempf et al. 2007) and producing males earlier than female sexuals is futile. Here, we investigate how variation in queen number affects the brood production pattern in two species with short-lived, peaceful, winged males and short-lived, wingless fighter males, Cardiocondyla minutior and Cardiocondyla obscurior. From LMC theory we predicted that wingless males should be reared earlier in multi-queen than in single-queen colonies as in Cardiocondyla sp. A. However, owing to the shorter life expectancy of males, males should not be produced earlier than female sexuals. Furthermore, from the proximate mechanisms of morph determination (e.g. Cremer & Heinze 2003) we expected that within a colony wingless and winged males should be produced at the same time, even though LMC affects winged males less than wingless males.

2. MATERIAL AND METHODS

Cardiocondyla obscurior and C. minutior are tramp ants, which have been introduced accidentally into man-made habitats throughout the tropics and subtropics, including South America (Heinze et al. 2006). Both species normally produce wingless fighter males, but switch to the production of winged males under stressful environmental conditions, such as drastic changes in colony size or temperature drops (e.g. Cremer & Heinze 2003; Heinze et al. 2004; Schrempf & Heinze 2006; Du et al. 2007). While wingless fighter males stay and mate in the maternal nest, winged males are docile, mate with female sexual nestmates during the first days of their adult lives and later disperse. They are usually not attacked because at least in C. obscurior they mimic the odour of female sexuals (Cremer et al. 2002). Queens rarely found new colonies independently. Instead, groups of workers, queens and brood may leave the maternal nest and settle in a nest site nearby in a process called ‘budding’ (Heinze & Delabie 2005).
3. RESULTS
First eggs were laid slightly later in two-queen than single-queen colonies of *C. minutior* (median, quartiles: two-queen colonies 16, 14 and 19 days; single-queen colonies 14, 13 and 15 days; median test, $\chi^2=0.394, p=0.047$). No difference was observed in the timing of the appearance of first larvae (median, quartiles: two-queen colonies 22, 21 and 25 days; single-queen colonies 22, 20 and 27 days, $\chi^2=0.158, p=0.691$) and first worker pupae (two-queen colonies 40, 39 and 47 days; single-queen colonies 42, 39 and 49 days, $\chi^2=0.158, p=0.691$).

Wingless male pupae were produced significantly earlier in multi-queen than in single-queen colonies of both species (figure 1). Similarly, female sexual pupae and, in *C. minutior*, also winged male pupae were reared significantly earlier in multi-queen than single-queen colonies. The difference between single- and multi-queen colonies was the strongest concerning the timing of the production of wingless males of *C. minutior* (difference between medians: 38 days; in *C. obscurior* 19 days, in all other sexuals less than 15 days). In none of the *C. minutior* colonies but in a considerable percentage of *C. obscurior* colonies (one of six single-queen colonies, 6 of 18 multi-queen colonies) males were produced before the first worker pupae. Males (regardless of morphology) were not produced earlier than young queens (sign tests: *C. minutior*, single queen: $z=0.289, p=0.773$; multi-queen: $z=0.316, p=0.752$; *C. obscurior*, single queen: $z=0.408, p=0.683$; multi-queen: $z=0.316, p=0.752$).

4. DISCUSSION
Variation in the number of queens in colonies of *C. obscurior* and *C. minutior* influences the timing of the emergence of sexuals. As expected from LMC theory, and similar to *Cardiocondyla* sp. A (Yamauchi et al. 2006), males were produced earlier in multi-queen than in single-queen colonies, but the details of brood production differ strikingly. Males of *Cardiocondyla* sp. A are extremely long-lived and in multi-queen colonies are produced long before female sexuals and regularly also before first workers emerge (Yamauchi et al. 2006). By contrast, wingless and winged males of *C. minutior* and *C. obscurior* are relatively short-lived. Consequently, the difference between single- and multi-queen societies in the appearance of first male pupae was less pronounced than in *Cardiocondyla* sp. A. Furthermore, queen number variation in *C. obscurior* and *C. minutior* also affected the production of female sexuals: in multi-queen colonies, both the first males and the first female sexuals were produced earlier than in single-queen colonies and at more or less the same time. Queens of *C. minutior* and *C. obscurior* thus achieve a similar outcome with short-lived males as queens of...
Cardiocondyla sp. A with long-lived males, i.e. an increased probability that their own sons survive fights with competitors and mate. Early production of female sexuals might in addition reflect competition among mother queens, which in this way increase the likelihood of their daughters being readopted into the nest. Cardiocondyla obscurior queens react to the presence of other queens by producing a less female-biased sex ratio in their eggs (Cremer & Heinze 2002; De Menten et al. 2005). The earlier production of males in multi-queen colonies of C. obscurior might thus in part reflect the higher likelihood that eggs remain unfertilized and are male destined. However, an altered primary sex ratio alone is probably not sufficient to explain the full magnitude of the change in the production pattern. Furthermore, though sex ratios of C. minutior varied much less with queen number (Heinze et al. 2004), queens were capable of adjusting the timing of the production of unfertilized eggs, suggesting that the timing and magnitude of male production are not strictly associated.

Predictions from LMC theory do not hold for winged males, which disperse a few days after emergence to mate away from the maternal nest (e.g. Cremer et al. 2002). Nevertheless, multi-queen colonies of C. minutior produced winged males significantly earlier than single-queen colonies and a similar, though non-significant, trend was observed in C. obscurior. This probably reflects the proximate mechanisms underlying male morph differentiation. Male morphology is not genetically fixed but is determined by workers in response to environmental stress (e.g. Cremer & Heinze 2003). Establishing new experimental colonies regularly provokes the production of winged males. When queens lay haploid eggs earlier in response to the presence of other queens, and workers react to environmental conditions, queen number affects the production of both winged and wingless males. The disparity between C. obscurior and C. minutior can be explained by different experimental conditions: C. obscurior queens had already been fertile for an unknown time, while C. minutior queens mated at the beginning of the experiment. This presumably also underlies the difference in the timing of the first appearance of pupae between the two species. However, as all adults were taken from stock colonies with similar composition, previous experience cannot explain differences between conspecific colonies.

Our study shows that variation in queen number in ants may not only affect sex allocation but also the timing of the emergence of both male and female sexuals. It therefore adds an intriguing new facet to the mechanisms of conflict and conflict resolution in social insects.

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