



Primary sex ratio adjustment by ant queens in response to local mate competition

LUDIVINE DE MENTEN*, SYLVIA CREMER†, JÜRGEN HEINZE† & SERGE ARON*

*Behavioral and Evolutionary Ecology, Université Libre de Bruxelles

†Biology 1, University of Regensburg

(Received 4 June 2004; initial acceptance 19 August 2004;
final acceptance 8 September 2004; published online 3 February 2005; MS. number: 8155)

In the ant *Cardiocondyla obscurior*, wingless males compete with nestmate males for access to female mating partners, leading to local mate competition (LMC). Queen number varies between colonies, resulting in variation in the strength of LMC. Cremer & Heinze (2002, *Proceedings of the Royal Society of London, Series B*, **269**, 417–422) showed that colonies responded to increasing queen number by producing a less female-biased sex ratio, as predicted by LMC theory. However, the proximate mechanisms responsible for this variation in the sex ratio could not be determined because the study was restricted to adult sex ratios. With LMC, the primary sex ratio (proportion of haploid eggs laid by the queen) is expected to be female biased, which lowers the conflict between queens and workers over sex allocation. We compared the primary sex ratios laid by queens in monogynous and in polygynous experimental colonies of *C. obscurior*. The proportion of haploid eggs laid by queens was significantly lower in single-queen than in multiple-queen colonies. Furthermore, queens rapidly adjusted their primary sex ratios to changes in colony queen number. This is the first report of an adaptive adjustment of the primary sex ratio in response to LMC by ant queens.

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Local mate competition (LMC), i.e. the competition among related males for access to females in a locally restricted area, is considered to be unusual in eusocial Hymenoptera (ants, bees, wasps; Crozier & Pamilo 1996). In ants, it has been reported in only six species: two parasitic ants (*Myrmoxenus krausseii*: Winter & Buschinger 1983; *Plagiolepis xene*: Aron et al. 1999) and four free-living species (*Messor aciculatus*: Hasegawa & Yamaguchi 1995; *Technomyrmex albipes*: Tsuji & Yamauchi 1994; *Myrmica sulcinodis*: Pedersen & Boomsma 1998; *Cardiocondyla obscurior*: Cremer & Heinze 2002). The proximate mechanisms responsible for the female bias under LMC in ants remain unknown. Sex ratio determination in eusocial Hymenoptera depends on two parties, the laying queens and the brood-rearing workers. Studies have shown that sex allocation may be influenced (1) by variation in the primary sex ratio (i.e. the proportion of haploid-male

eggs) laid by the queens, (2) by workers manipulating the colony secondary sex ratio (i.e. the proportion of males among adult sexuals) by selectively eliminating male brood and/or preferentially feeding females, or (3) by a combination of both (Chapuisat & Keller 1999; Passera et al. 2001; Sundström & Boomsma 2001; Beekman & Ratnieks 2003).

The ability of queens and workers to bias colony sex ratios in social Hymenoptera has been mostly studied in the framework of the queen–worker conflict over sex allocation, with workers usually preferring a more female-biased brood than queens (Trivers & Hare 1976; Bourke & Franks 1995; Crozier & Pamilo 1996). Under LMC, however, the respective sex allocation optima of queens and workers converge towards a female-biased brood, because the fitness return from males decreases for both parties (Bourke & Franks 1995). In species experiencing LMC, the primary sex ratio laid by the queens is therefore expected to be female biased and no further manipulation of the sex ratio by the workers during brood rearing should occur. Consistent with this prediction, the female-biased sex ratio associated with LMC has been shown to result from queens producing a female-biased primary sex ratio in the workerless parasitic ant *P. xene* (Aron et al. 1999). However, this species has lost the

Correspondence: S. Aron, Behavioral and Evolutionary Ecology – CP160/12, Université Libre de Bruxelles, av. F.D. Roosevelt, 50, B-1050 Brussels, Belgium (email: saron@ulb.ac.be). S. Cremer is now at the Department of Population Biology, Biological Institute, University of Copenhagen, DK-2100 Copenhagen O, Denmark. J. Heinze is at Biology 1, University of Regensburg, Universitätsstrasse 31, D-93040 Regensburg, Germany.

worker caste so that the female-biased investment could not stem from workers manipulating the sex ratio. Furthermore, the ability of the queens to adjust the primary sex ratio in response to different intensities of LMC was not investigated.

We investigated the primary sex ratio laid by queens in response to varying strength of LMC in the ant *C. obscurior*. This species shows a male dispersal dimorphism, with winged males specialized in dispersal and wingless, ergatoid 'fighter' males specialized in mating inside the nest (Kinomura & Yamauchi 1987; Stuart et al. 1987). Ergatoid males never leave the nest and engage in mortal combat with their rivals to mate with the virgin queens produced in their natal colony. In *C. obscurior*, the number of nestmate queens may change rapidly because of adoption of new queens or merging or splitting of colonies. Colony-level sex ratio varies in response to the strength of LMC resulting from varying queen number (Cremer & Heinze 2002). In accordance with the predictions of the LMC theory, single-queen (monogynous) colonies produce fewer ergatoid males and a more female-biased sex ratio than multiple-queen (polygynous) colonies. Cremer & Heinze could not determine whether these variations in the sex ratio stem from queens adjusting their primary sex ratios or from workers manipulating the secondary sex ratios in response to LMC because the study was restricted to adult sex ratios. In this study, we compared the primary sex ratios in monogynous colonies, in which a single queen produces all males, and polygynous colonies, where many queens produce male offspring. Owing to the lower relatedness among nestmate males, LMC should be relaxed in the latter situation.

METHODS

Ant Colonies

Eight polygynous colonies of *C. obscurior* were collected in Bahia, Brazil and cultivated in the laboratory as described elsewhere (Heinze et al. 1998). Permission for collection and export was given by the Brazilian Minister of Science and Technology. No licence was required for import. Each colony was split into one colony fragment containing a single queen (monogynous) and another fragment containing five queens (polygynous), all with an initial number of 20 workers per colony fragment. This number of workers corresponds to the median number in natural nests (median = 20, maximum = 81, $N = 57$ colonies), and is sufficient for regular production of sexuals (which starts when colonies have a minimum of about 10 workers, S. Cremer, personal observation). Workers of *Cardiocondyla* are completely sterile (Heinze et al. 1993; Seifert 2003), so that all eggs laid in a colony come from the queens.

After 1 month, we removed all eggs present in the colony fragments. The eggs laid during the next 10 days were then collected from each fragment and stored in a freezing milieu (10% DMSO/Basal Medium Eagle) at -80°C for subsequent cytogenetic analyses. Our egg sample therefore comprised almost exclusively eggs laid

4.5–6 weeks after colony fragments were set up. Immediately after collecting the eggs, we split the polygynous colony fragments into several monogynous subunits, each with a minimum number of 20 workers (with additional workers from the respective source colony when necessary). These monogynous subunits are hereafter referred to as 'Poly > Mono' fragments. The eggs laid 4.5–6 weeks after the Poly > Mono fragments were set up were collected and stored in the freezing milieu. Thus, the experiment lasted 12 weeks, with monogynous and polygynous fragments being set up at the same time (first 6 weeks) and the second experiment Poly > Mono in the second 6 weeks. To obtain a reliable estimate of the primary sex ratio, we did not include queens producing under 20 eggs or that died during the experiment. Overall, our sample consisted of eggs from seven monogynous colony fragments, eight polygynous colony fragments and nine Poly > Mono colony fragments. Four of the nine Poly > Mono fragments originated from the same polygynous colony and were therefore not independent.

Primary Sex Ratio Determination

We determined the ploidy-level (haploid or diploid) of the eggs by FISH (Fluorescence in situ Hybridization), by following the procedure of de Menten et al. (2003). A 4.5-kbp sequence of the *abd-A* gene from the ant *Myrmica rubra* was used as a unique DNA-probe. The probe was labelled with biotin and detected with an avidin-conjugated FITC fluorophore. Ploidy-level determination was based on the number of fluorescent spots per nucleus. Several nuclei were observed for each egg ($\bar{X} \pm \text{SD} = 8.5 \pm 2.5$; range 4–16). The number of eggs for which we could determine ploidy-level varied between 9 and 26 ($\bar{X} \pm \text{SD} = 17.8 \pm 4.0$) per sample. All primary sex determinations were realized blindly with regard to treatment, i.e. the research worker did not know which colony was monogynous or polygynous during egg analyses.

Statistical Analysis

Comparison of the proportion of haploid eggs laid by queens between monogynous and polygynous fragments derived from the same field colonies indicated that pairing was not effective (nonparametric Spearman correlation: $r_s < 0$ for all comparisons). Data from polygynous and monogynous fragments were therefore considered as independent data. A logistic binomial regression model was carried out using the procedure GENMOD in SAS (version 8.2, SAS Institute Inc., Cary, North Carolina, U.S.A.) to explore the effect of colony type entered as a fixed factor (with three types considered: monogynous, polygynous and Poly > Mono) on their sex ratio (Boomsma & Nachman 2002). This type of statistical model assumes that the dependent variable is a probability that follows a binomial distribution, and allows us to take variations in clutch size into account. The sex ratio was entered in the model as the dependent variable, and colony type as a qualitative variable. There was no need to adjust the model scale

parameter as it revealed no evidence of overdispersion ($s = \sqrt{D/v} = 0.59$, where D is the deviance and v is the degrees of freedom).

RESULTS

There was a significant difference in the primary sex ratio laid by queens from monogynous and polygynous fragments (logistic regression analysis: $\chi^2_1 = 4.66$, $P = 0.031$). Whereas the average proportion of haploid eggs laid by queens in single-queen colony fragments \pm SD was 0.08 ± 0.04 (range 0.05–0.14, $N = 7$), this value increased to 0.18 ± 0.07 (range 0.05–0.25, $N = 8$) in multiple-queen fragments (Fig. 1). When these polygynous fragments were split into monogynous subunits for an additional 6 weeks (i.e. Poly > Mono fragments), the proportion of haploid eggs laid by queens decreased to 0.09 ± 0.05 (range 0.0–0.14, $N = 6$), a value significantly different from that in the original polygynous fragments ($\chi^2_1 = 4.93$, $P = 0.026$) but similar to that laid in the first set of monogynous ones ($\chi^2_1 = 0.01$, $P = 0.917$). For one of the polygynous fragments investigated, four queens were analysed 6 weeks after fragmentation into monogynous subunits. All produced similarly low proportions of haploid eggs (0.0, 0.0, 0.08 and 0.09) compared to previously polygynous conditions (0.21).

DISCUSSION

This study shows that queens of the ant *C. obscurior* lay a significantly lower proportion of haploid eggs in single-queen than in multiple-queen colonies. The adjustment of the primary sex ratio in response to the changing conditions in the colony (presence/absence of other queens) was relatively rapid, as it occurred within 6 weeks.

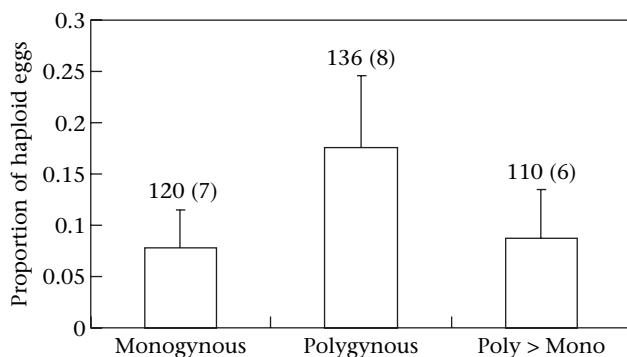


Figure 1. Primary sex ratio expressed as the numerical proportion of haploid eggs ($\bar{X} \pm$ SD) laid by queens of monogynous, polygynous and Poly > Mono experimental colonies in the ant *C. obscurior*. The total number of eggs and the number of colonies from which they were collected (in parentheses) are reported above the bars for each condition. A single value is given for the four Poly > Mono fragments originating from the same polygynous colony (see Methods). Twenty eggs were randomly selected from a total of 82 eggs laid by the four queens. The procedure was repeated 100 times and the mean proportion of haploid eggs obtained (0.10) was considered in the analysis.

These results are consistent with the predictions of the LMC theory, because males in monogynous colonies are more closely related and experience a higher local competition for mates than males in polygynous colonies. Thus, queens reduce the proportion of haploid eggs laid when the relatedness among the male offspring, which will compete locally for mates, increases in the colony. This finding confirms Cremer & Heinze's (2002) suggestion that queens of *C. obscurior*, but not workers, adjust colony-level sex allocation in response to the number of queens present in the nest. Cremer & Heinze altered the genetic composition of the brood of monogynous colonies by transferring eggs from alien 'donor' monogynous or polygynous colonies. The secondary sex ratios obtained were then compared with those expected from a theoretical framework, where queens, workers or both parties were in control of sex allocation. Their experimental data were in accordance with queen control of the sex allocation. However, their study was based on variations in the secondary sex ratio only. Whether queens adjusted primary sex ratio in response to the number of queens in the colony and, hence, to the strength of LMC was not directly investigated. To our knowledge, our study is the first to provide direct evidence for adaptive biasing of the proportion of haploid eggs in response to LMC in a social Hymenopteran.

The possibility that our estimates of the primary sex ratio could be biased by workers preferentially cannibalizing eggs of one sex seems unlikely. Egg cannibalism in *C. obscurior* was never observed in the many hours of observations (S. Cremer, unpublished data). Furthermore, no study has shown that queen-laid male eggs are treated differently to queen-laid female eggs. Rather, in several ant species, workers perform sex allocation biasing by killing male larvae but not eggs (Passera & Aron 1996; Chapuisat & Keller 1999; Aron et al. 2004).

The adaptive fine-tuning of primary sex ratios through selective fertilization of eggs under LMC has been widely documented in solitary Hymenoptera. For example, females of the parasitoid wasp *Nasonia vitripennis* adjust the sex ratio of their brood in response to the presence of eggs from other females within a host (Werren 1980, 1983). The first female to lay eggs produces a strongly female-biased sex ratio, while the second female adjusts the proportion of sons to the relative level of LMC, as predicted by the natural selection model. Previous experiments (Cremer & Heinze 2002) suggest that queens of *C. obscurior* could use the actual number of reproducing queens in their colony, rather than the presence of brood from other females, as an informative cue to estimate colony structure.

Our data on the primary sex ratio laid by queens reared in monogynous (0.08) and polygynous colony fragments (0.18) may be compared with those previously obtained on the secondary sex ratio in the same experimental conditions, where the proportions of hatching males among sexuals were, respectively, 0.35 and 0.62 (Cremer & Heinze 2002). A higher percentage of males is expected in the secondary sex ratio since it does not include workers, which are counted as diploid eggs in primary sex ratios. Unfortunately, data on the production of new workers was not available in this study. Nevertheless, both

the proportion of haploid eggs and the proportion of adult males were approximately doubled under polygyny versus monogyny. This strongly suggests that workers do not significantly bias the secondary sex ratio during brood development according to the strength of LMC. Workers may, however, affect the determination of male morph, because male dimorphism (ergatoid versus winged) in *C. obscurior* is not genetically determined, but is induced by environmental conditions (temperature, colony size and probably resource availability) during larval development (Cremer & Heinze 2003). Under good environmental conditions, colonies invest almost exclusively in ergatoid males; when conditions turn bad, they start to produce winged males. Similarly, Cremer & Heinze (2002) showed that the higher proportion of adult males produced under polygyny (compared to monogyny) stemmed from an increase in the proportion of ergatoid males reared at the expense of the number of female sexuals. In contrast, there was no difference in the proportion of winged males produced between both colony types. Workers of *C. obscurior* therefore rear a constant proportion of winged males and the remaining part of the haploid brood become wingless males.

The ability of reproductive females to bias the primary sex ratio in response to ecological and/or social conditions that differentially affect the fitness of sons and daughters in eusocial Hymenoptera remains poorly studied (Ratnieks & Keller 1998). In ants, queens can adjust the primary sex ratio according to the life history stage of their colony (Aron & Passera 1999), seasonal variations (Aron et al. 1994; Keller et al. 1996a) and the competition among queens over male parentage (Keller et al. 1996b). Our data show that they can also adaptively adjust the primary sex ratio in response to local mate competition.

Acknowledgments

We thank J. S. Pedersen, M. Chapuisat, S. West and two anonymous referees for improving the manuscript. Thanks also to M. Gilbert for his advice with the statistical part of the work. Financial support was provided by the FRIA (L.M.), the DFG (Deutsche Forschungsgemeinschaft: He 1623/12-2) and a Feodor Lynen Fellowship by the Alexander von Humboldt-Foundation (S.C.), and the Belgian National Fund for Scientific Research (Conventions: 2.4597.05, 2.4569.02, 15.174.04) (S.A.).

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