

Reproductive conflicts in cooperative associations of fire ant queens (*Solenopsis invicta*)

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SUMMARY

In ants, unrelated queens frequently associate to initiate a colony cooperatively. The joint reproductive effort of the cofoundresses increases growth and survival of the incipient colony. However, such associations are unstable. Soon after emergence of the first workers, queen–queen and queen–worker fights lead to the death or expulsion of all but one cofoundress. Because no sexual offspring are produced in incipient colonies the surviving queen monopolizes the entire future reproductive success of the colony. Two factors, the queens' relative fighting ability and their relative contribution to worker production (assuming that workers can recognize and selectively favour their mother) have been proposed to influence the survival prospects of individual queens within associations. The effect of these two factors was tested in the fire ant *Solenopsis invicta*. Initial size differences, a potential measure of the queens' fighting ability, affected the outcome of the conflicts, so that the initially heavier queen was more likely to survive. Differential loss of mass by initially equal nestmates also affected survival, with the queen losing more body mass being more likely to die. The manipulation of the queens' relative contribution to the worker brood did not significantly affect the queens' survival probability, suggesting that workers are unable to favour their mother.

1. INTRODUCTION

Individual-level selection leads to reproductive conflicts among group members within genetically heterogeneous animal societies (Hamilton 1964). Colony founding by queen associations in ants provides a good model to investigate the factors affecting the outcome of reproductive conflicts in cooperative groups. Mature colonies periodically release sexual offspring on mating flights. In most species, after mating queens found colonies independently, i.e. without the help of workers. The queen typically seals herself in a burrow and rears her first worker brood using her metabolic energy stores (Markin *et al.* 1972; Porter *et al.* 1988; Keller & Passera 1989). The founding of new colonies is a critical stage in the life cycle and most incipient nests perish before the first workers have a chance to forage (Tschinkel 1992). In the fire ant *Solenopsis invicta* (Markin *et al.* 1972), as in many other ant species (Bartz & Hölldobler 1982; Rissing & Pollock 1988; Strassmann 1989), queens can either embark on colony founding alone (haplometrosis) or join other unrelated queens to initiate a new colony (pleometrosis). Pleometrotic associations show higher survival through the early phase of colony founding and produce a larger worker brood. The workers of the first cohort often steal brood from neighbouring nests. This phenomenon, known as brood raiding, is a major source of colony mortality in *S. invicta* (Tschinkel 1992) and other pleometrotic ant species (Rissing & Pollock 1988). Colonies starting with a larger worker force are

usually more successful in raiding brood from neighbouring colonies (Rissing & Pollock 1988; Adams & Tschinkel 1995). Furthermore, colonies founded through pleometrosis produce sexual offspring earlier than colonies founded by a single queen (Vargo 1988).

However, whereas pleometrosis is a form of insurance for the colony, it is a high-stakes gamble for the individual queen (Seger 1993). The eclosion of the first workers elicits the outbreak of overtly aggressive behaviour among colony members (Rissing & Pollock 1988; Sommer & Hölldobler 1995). Queen–queen and queen–worker fights result in the execution or expulsion of all but one queen soon after worker emergence. The surviving queen monopolizes the entire future reproductive success of the colony as no sexual offspring are produced at this early stage. Reduction of queen number to a single queen is the rule in pleometrotic associations of fire ants and of other ant species as well (Heinze 1993).

Little is known about which factors affect the competitive ability and the survival prospects of individual queens in pleometrotic associations. Queen–queen fights are frequently observed with bouts lasting up to more than one hour (unpublished data). Workers also may take part to the fights, the attacks being frequently directed against already injured and mutilated queens. In this study, we examined two factors which have been proposed to influence survival prospects of queens (Reeve & Ratnieks 1993). If the size of queens is correlated with relative competitive ability (as for example in the case of *Polistes* foundresses,

reviewed by Reeve 1991), then the heavier queen at worker eclosion is predicted to have a higher chance of winning the fights. Alternatively, if workers are able to selectively favour their mother during fights or through preferential feeding, the queen that produced the higher number of workers is expected to have higher survival prospects than her nestmate. The workers might recognize their mother either by using genetic cues or, alternatively, by favouring the most productive queen as an effective rule of thumb for identifying their mother queen (Forsyth 1980).

Differences in mass at the time when fights initiate can result from initial differences in mass or differential loss of during colony founding. We tested whether initial differences in mass and differential loss of mass predict the outcome of the fights in separate experiments. To test whether workers can recognize their mother and behave in a nepotistic way to increase her probability of survival we manipulated the queens' relative contribution to brood production.

2. MATERIAL AND METHODS

Queens were collected from monogynous *S. invicta* populations in Georgia and Florida immediately after mating flights in early summer 1994 and 1995. Mature colonies contain a single queen in monogynous populations (Ross & Fletcher 1985a) and colony founding occurs as described above. Field surveys revealed that approximately half of the colonies contained more than one queen, with pairs of queens being the most common type of pleometrotic associations (figure 1, after Tschinkel & Howard 1983). During the first three weeks, queens oviposit and take care of the developing brood. The first workers eclose after day 21 and queen execution occurs in week 4–6 from the beginning of colony founding (Markin *et al.* 1972, 1973; Voss & Blum 1987). Individuals were shipped immediately to the laboratory where they were placed in a nest within 48 h hours of collection when oviposition is about to start (Markin *et al.* 1972; Voss & Blum 1987). Queens used in any given experiment were from the same mating flight.

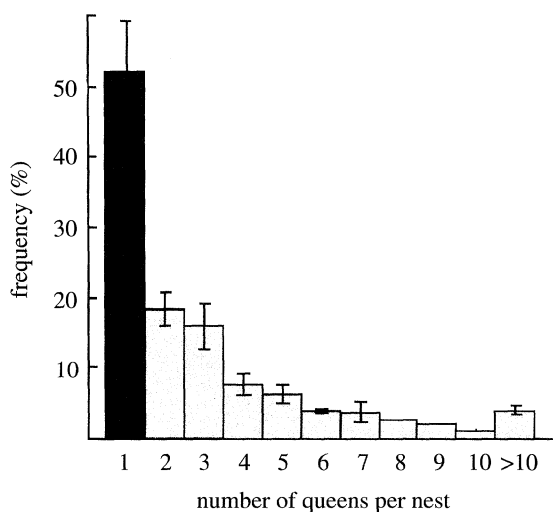


Figure 1. Frequency distribution of *S. invicta* queen associations of different sizes. Means (\pm s.e.) are calculated from data of seven mating flights (after Tschinkel & Howard 1983). Black bar: single-queen nests; shaded bars: pleometrotic nests.

The factors potentially affecting queen survival were investigated in two-queen colonies. Queens were paired according to their mass on day 1 (nestmates were of the same initial mass in all experiments except in one, see below). Queens were weighed to the nearest 0.1 mg. The nest consisted of a petri dish (diameter 35 mm) glued in the centre of a foraging arena (petri dish, diameter 80 mm) coated with Fluon to prevent escape. Workers but not queens had access to the foraging arena through a hole (diameter 1.5 mm). Nests were placed on wet cotton in plastic trays. A hole (diameter 4.8 mm) through the bottom of the nest, filled with a mixture of dental plaster and plaster of Paris, allowed moisture to diffuse into the nest. A plaster rim along the internal edge of the nest provided a dry substrate for the brood. Colonies were kept at 29 ± 2 °C, 70% relative humidity. Queens were marked on the thorax with paint markers PX200. Colonies were fed immediately after the eclosion of the first workers with beef meat (baby food) every other day. After worker emergence colonies were observed daily until queen execution.

To test whether relative loss of mass during colony founding predicts the outcome of queen–queen conflicts we paired 180 queens of the same initial mass. Queens were weighed again on day 10 and 20 after pairing to quantify differential loss of mass between nestmates.

To test whether initial size differences affect survival prospects we weighed 120 queens and ranked them according to mass. Queens were then paired in 60 colonies as follows. The heaviest queen was paired with the 61st heaviest queen, the second heaviest with the 62nd queen, etc. This procedure ensured that the differences in mass between nestmates were similar in all colonies (average difference 1.5 ± 0.3 mg, $N = 60$).

To test whether queen survival depends on their relative contribution to worker production, we manipulated the share each queen had in the developing brood. In a first experiment queens were kept singly until day 7 at which time they were paired with another queen ($N = 60$). The new colony received the whole brood of only one (randomly chosen) of the two queens and only a few eggs of the other. The two-queen colony was given a fresh nest. The first worker cohort develops from the eggs laid during the first week (Voss & Blum 1987) thus the workers present at the time of queen fighting were virtually all of the same matriline, i.e. full sisters because *S. invicta* queens mate once (Ross & Fletcher 1985a). A few eggs of the other queen were added so that workers of the most frequent matriline were exposed also to a few unrelated workers, which might be necessary for discrimination learning (Buckle & Greenberg 1981). We conducted a second experiment similar to the first except that queens were kept alone until day 21 at which time two-queen colonies were constructed ($N = 50$) with only the brood of one of the queens. Hence, in this experiment not only workers, but also most older brood are the offspring of a single queen at the time when queen execution occurs.

For the analyses we considered only healthy colonies in which a single queen survived. We excluded colonies where both queens died on the same day, or within a few days when the survivor was heavily injured (17%), colonies which failed to produce adult workers (1%), colonies where queen death occurred before day 21 (i.e. before worker eclosion and the outbreak of fights, 17%), colonies in which both nestmates were still alive after day 45 or 65 (day of censoring varied between experiments, 7%), colonies where queens lost the paint mark (2%) and colonies containing diploid males (10%; overall 48% of the colonies were discarded). A high proportion of queens in US populations produce diploid males (Ross & Fletcher 1985b) as a result of the loss of alleles at the sex-determining locus due to a founder event (Ross *et*

al. 1993). Diploid male larvae are much larger than worker larvae and impose an energetic drain which usually results in the colony death (Ross & Fletcher 1986).

The number of observations is given by n , or N for the number of colonies. The Spearman rank correlation coefficient is indicated by r_s . Mean values are followed by \pm s.d. unless specified. Day 1 is the day when ants arrived at the laboratory and experiments were started.

3. RESULTS

Differential loss of mass by nestmate queens of the same initial mass significantly affected survival prospects. The survivors lost $36\% \pm 7\%$ of their initial mass between day 1 and day 20, whereas the losers lost $41\% \pm 8\%$ ($x \pm$ s.d.; Wilcoxon signed-rank test, $N = 41$, $z = -2.1$, $p = 0.03$). Survivors were already slightly heavier (12.3 ± 1.2 mg) than losers (11.9 ± 1.2 mg) on day 10, although the difference was not significant (Wilcoxon signed-rank test, $N = 41$, $z = -1.38$, $p = 0.17$) (figure 2). These colonies contained 26 ± 14 workers ($N = 37$, 4 missing values) at the time of queen execution, which occurred 35 ± 11 days ($N = 41$) after the beginning of the experiment.

The number of workers was significantly correlated with the combined loss of mass of the two nestmate queens between day 10 and day 20 ($N = 37$, $r_s = 0.46$, $p < 0.01$) but not with their combined initial mass ($N = 37$, $r_s = -0.12$, $p = 0.47$). The number of workers at queen execution was significantly correlated with the number of larvae present on day 14 ($r_s = 0.68$, $p < 0.01$, $N = 16$, brood counted in a subset of colonies) and the number of pupae on day 21 ($r_s = 0.80$, $p < 0.01$, $N = 16$), indicating that worker number is a reliable estimate of brood production during early colony founding.

Initial size differences between queens significantly affected their survival prospects (Wilcoxon signed-rank test for initial mass of survivors versus losers, $N = 20$, $z = -2.07$, $p = 0.039$), with the initially heavier queen surviving in 14 out of 20 colonies (70%). The initial difference in mass persisted throughout

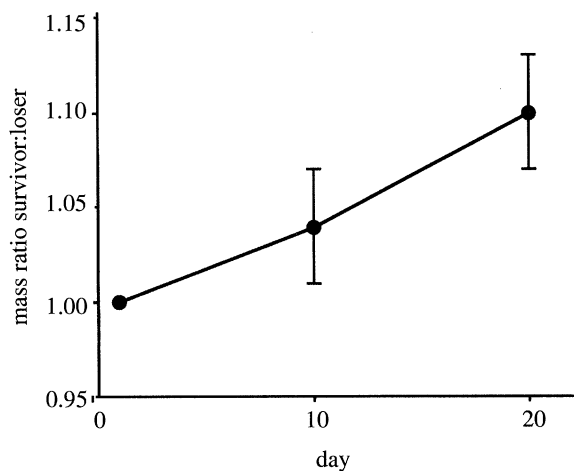


Figure 2. Relative mass of the surviving queen and her nestmate over the first 20 days of colony foundation ($x \pm$ s.e., $N = 41$ pairs of queens of same initial mass).

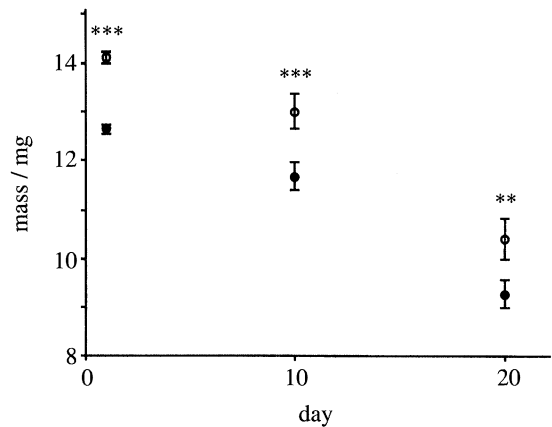


Figure 3. Mass of queens in two-queen colonies with unequal initial mass ($x \pm$ s.e., $N = 20$). Open circles: initially heavier nestmate; filled circles: initially lighter nestmate. Wilcoxon signed-rank test: ***, $p < 0.001$; **, $p < 0.01$.

colony founding in 79% of the colonies (15 out of 19, 1 tie; difference in mass on day 1 and day 20: 1.5 ± 0.3 mg and 1.1 ± 1.7 mg, respectively; figure 3). The time until queen execution tended to be shorter in colonies where the initially heavier queen survived (34 ± 8 , $N = 14$) than in colonies with the opposite outcome (39 ± 7 , $N = 6$; U-test, $z = -1.79$, $p = 0.07$).

Also in this experiment, the number of workers produced was not significantly correlated with the combined initial mass of queens ($N = 17$, $r_s = -0.16$, $p = 0.56$, 3 missing values). The correlation with the combined loss of mass was positive ($r_s = 0.38$) as in the experiment with pairs of queens of the same initial mass, although not significantly so ($N = 17$, $p = 0.13$). The lack of significance was probably due to smaller sample size.

The manipulation of the queens' relative contribution to the brood had no significant effect on the survival probability of queens. The queens which were transferred with their brood to the two-queen colonies on day 7 survived in 18 out of 31 colonies (58%) (binomial test, $p = 0.47$). Similar results were obtained in the second experiment where queens were combined on day 21. In 21 out of 42 colonies (50%) the queen transferred without progeny survived ($p > 0.5$), indicating that workers were unable to significantly increase their mother's probability of survival and that the absence of descendants of one queen among older larvae and pupae also had no significant effect.

4. DISCUSSION

The competitive ability of queens in pleometrotic associations of *S. invicta* was correlated with their mass. When colonies contained queens differing in initial mass the heavier queen was more likely to survive. Similar results have been obtained in *Lasius pallitarsis* (Nonacs 1990). In contrast, no significant effect of initial differences in mass between nestmate queens could be detected in *Lasius niger* (Sommer & Hölldobler 1995). The lack of a significant effect in *L. niger* may stem from interspecific differences in the factors

affecting competitive abilities of queens or from differences in the range of variation in mass tested experimentally. Queens differed by around 11% in our study and by 5% in the *L. niger* study.

Nonacs (1989) suggested that queens should preferentially join potential pleometrotic partners with lower competitive ability in ants. However, joining smaller queens may bear a cost in terms of reduced selective advantage of cooperative colony founding if smaller queens have lower energy reserves to invest in brood production thus reducing overall colony productivity. Our data showed no significant correlation between colony productivity and the combined initial mass of queens. Tschinkel (1993) obtained similar results for haplometrotic colonies. Thus, there is apparently no such cost to associate with small queens, supporting the view that smaller partners should be preferred if choice is possible. It is, however, unknown whether queens visit several burrows before associating with other queens under natural conditions, which likely incurs costs such as increased predation.

In the experiment where nestmate queens were of the same initial mass, the queen losing less weight relative to her nestmate was more likely to survive. This suggests that the competitive ability of queens is correlated with their physical condition at the time of fights. This conclusion is consistent with the finding that initially heavier queens were more likely to survive and that initial differences in mass between queens persisted in most colonies throughout colony founding. Models on partitioning of reproduction (Vehrencamp 1983; Reeve & Ratnieks 1993; Keller & Reeve 1994) generally assume that relative size correlates with competitive ability and our study supports this assumption. It remains to be tested whether the differences in loss of mass alone determine the outcome of conflicts and why one of the nestmates loses more.

The manipulations of the queens' relative contribution to the brood had no significant effect on queen survival. Queens whose worker offspring were present at the time of fights were not significantly more likely to survive than queens without daughter workers. A power analysis (Cohen 1988) of the combined results of the two experiments ($N = 73$) reveals that there was a 90% probability ($\alpha_1 = 5\%$) of detecting a deviation as small as 1/6 from the null hypothesis of random survival, i.e. a situation where the queen with daughter workers would survive in 67% of the cases.

Considerable controversy has arisen on whether workers can enhance their inclusive fitness by biasing altruistic behaviour in favour of their closer kin in genetically heterogeneous colonies (Page *et al.* 1989; Carlin & Frumhoff 1990; Oldroyd *et al.* 1990). Pleometrotic foundations of ants provide an extreme case in which workers may influence the probability of survival of kin (their mother) at the expense of non-kin (other queens in pleometrotic associations). Our finding that workers do not significantly enhance their mother's probability of survival adds to the increasing body of evidence that within-colony kin recognition and discrimination are rare or absent in social insects (Carlin & Frumhoff 1990; Oldroyd *et al.* 1990; Queller *et al.* 1990; Carlin *et al.* 1993; Bourke 1994). Nepotism

can reduce colony efficiency, which may act as a brake for the evolution of kin-discrimination behaviour (Reeve 1989; Carlin *et al.* 1993). This constraint might apply in particular to early stages of colony development that are under strong selective pressure for rapid growth, as is the case for early colony foundations in ants.

In conclusion, this study supports the hypothesis that competitive abilities of queens in cooperative associations of fire ants are predicted by their relative initial size and their relative loss of mass during colony founding, with the heavier queen being more likely to survive. There was no evidence that workers are able to recognize and favour their mother, suggesting that the relative contribution of queens to worker production is not an important factor affecting their probability of survival.

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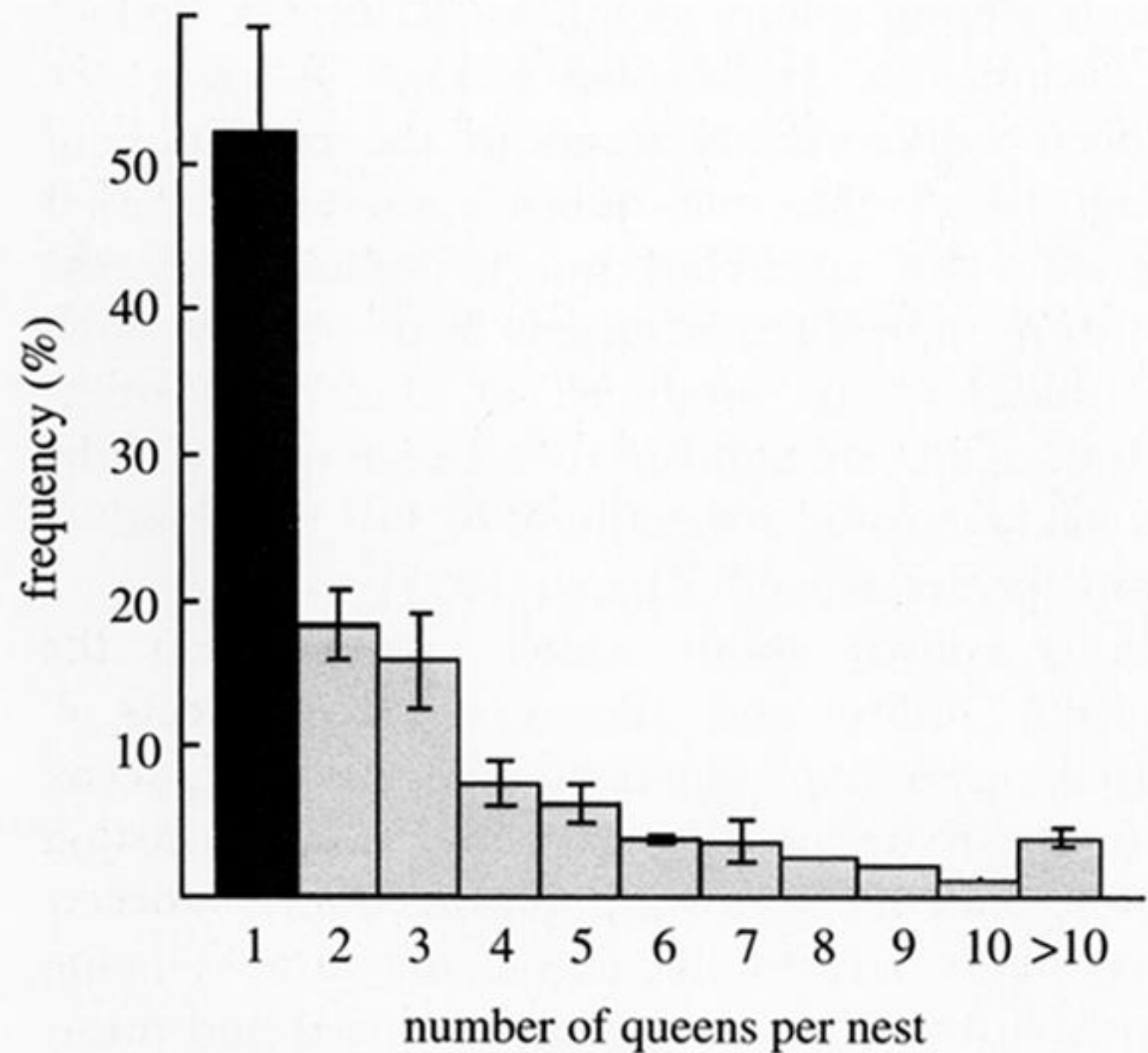


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