

Research article

Stay or drift? Queen acceptance in the ant *Formica paralugubris*

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Received 8 April 2008; revised 16 June 2008; accepted 1 July 2008.
Published Online First 18 July 2008

Abstract. The acceptance of new queens in ant colonies has profound effects on colony kin structure and inclusive fitness of workers. Therefore, it is important to study the recognition and discrimination behaviour of workers towards reproductive individuals entering established colonies. We examined the acceptance rate of queens in populations of the highly polygynous ant *F. paralugubris*, where the genetic differentiation among nests and discrimination ability among workers suggest that workers might reject foreign queens. We experimentally introduced young queens in their natal nest and in foreign nests. Surprisingly, the survival rate of mated queens did not differ significantly when introduced in a foreign male-producing nest, a foreign female-producing nest, or the natal nest. Moreover, the survival of virgin queens in their natal nest was twice the one of mated queens, suggesting that mating status plays an important role for acceptance. The results indicate that other factors than queen discrimination by workers are implicated in the limited long-distance gene flow between nests in these populations.

Keywords: Queen acceptance, mating status, dispersal, ants.

Introduction

In most social insects, including many ant species, individuals aggressively reject unrelated non-nestmates. This defence helps to maintain territorial colony boundaries, prevents the invasion and exploitation of the colony by unrelated conspecifics and ensures that altruistic acts are directed towards relatives (Hamilton, 1964; Crozier

and Pamilo, 1996). An increasing number of studies have investigated the discrimination ability between ant workers, but few have addressed the recognition and discrimination behaviour of workers towards reproductive individuals entering colonies. These studies are important, because accepting new queens will generally have a large impact on colony kin structure and inclusive fitness of workers (Heinze and Keller, 2000).

Ant colonies typically produce hundreds or thousands of new queens and only a tiny fraction will ever succeed in founding a new colony independently after the mating flight (Hölldobler and Wilson, 1990). In species with multiple queens per colony (polygynous species), alternative options for young queens are to stay and mate within the natal nest or to seek adoption in an established colony after the mating flight. Established colonies benefit from re-accepting queens when these new queens increase colony survival or productivity sufficiently to compensate for the decrease in inclusive fitness, or when the queens are related to the resident colony members (Nonacs, 1988). Nestmate queens are indeed generally related in many polygynous species, which confirms that queens often mate and stay in their natal nest, or return to their natal nest after the mating flight (Bourke and Franks, 1995; Keller, 1995; Chapuisat and Keller, 1999).

The process of queen replacement in social insects has received much attention (Heinze and Keller, 2000). In the queen replenishment hypothesis, Brown and Keller (2000) proposed that the production of new queens occurs preferentially or only in nests with relatively few queens. These are the nests that benefit most from recruiting new queens to enhance colony survival and productivity. But female-producing colonies appear to produce more queens than the ones needed for optimum productivity and survivorship. Brown and Keller (2000) suggested that the overproduction of queens is a way to dilute the effect of potentially accepting foreign queens during periods of queen replenishment. If workers are

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unable to distinguish between foreign and nestmate queens, they might avoid parasitism by foreign queens by killing all young queens in years when the colony does not produce queens (Rosengren and Pamilo, 1983; Brown and Keller, 2002).

Behavioural studies on queen adoption in polygynous ants report mixed results, ranging from total acceptance to complete rejection of foreign queens. In *F. exsecta* newly emerged virgin queens were more likely to be accepted in female-producing colonies, and were mostly killed in colonies that produced only males (Brown et al., 2003). A study in *F. truncorum* suggests that the mating status of queens influences their acceptance. Specifically, workers in laboratory colonies did not distinguish between nestmate and non-nestmate queens, but were more likely to accept virgin than mated queens (Sundström, 1997). In *F. polyctena*, all old foreign and mated queens were accepted (Rosengren et al., 1986). A recent laboratory study in the ponerine ant *Pachycondyla luteipes* showed that mated and reproductive active queens were accepted in their nest of origin, whereas 70% of the non-nestmate queens were rejected (Kikuchi et al., 2007). Similarly, in the Argentine ant *Linepithema humile* nestmate queens were generally accepted (90%), but most of the queens introduced into foreign colonies were killed (Vásquez and Silverman, 2008). Finally, in *Leptothorax curvispinosus* all foreign queens were rejected (Stuart et al., 1993), and in *Myrmica tahoensis* the acceptance rate of old queens was about 23% (Evans, 1996).

Our study species *F. paralugubris* has an extraordinary social structure with almost no aggression between nests that can contain as many as several hundreds of reproductive queens (Cherix, 1980, 1983). New sexuals are produced only once a year in spring and the mating flight takes place over two to three weeks in June. Young queens have alternative reproductive strategies (Cherix et al., 1991). They can mate and stay within the nest (Chapuisat and Keller, 1999). They can also fly to mating places and later join the natal or another established colony in the population, or found a new colony by parasitizing species of the subgenus *Serviformica* (Kutter, 1969; Cherix et al., 1991). Experimental introductions of queens in female-producing nests showed that virgin queens were accepted with a higher probability than mated queens, and suggested that the survival of introduced queens did not differ between natal and foreign nests (Fortelius et al., 1993). Although interesting, the results of this pioneering study are not fully conclusive. First, they suffer from pseudoreplication, as nests were used several times and the introduced queens originated from only three nests. Second, the duration of introduction was short (two hours) and no queen was introduced in male-producing nests, so that some discrimination might still occur over longer time period or in other types of nests.

A further investigation of queen acceptance was prompted by new genetic and behavioural data suggesting that workers might reject foreign queens. Distant

nests of our study population are genetically differentiated, which results in low but significant relatedness among nestmate workers, despite the high number of reproductive queens (Chapuisat et al., 1997; Chapuisat and Keller, 1999). This genetic differentiation and isolation by distance indicate that long-distance gene flow between established nests is limited and that most queens mate and stay within their natal nest (Chapuisat et al., 1997; Chapuisat and Keller, 1999). Such a lack of long-distance gene flow is surprising, because many queens fly and mate away from the nests (Cherix et al., 1991; Chapuisat, 1998). Another intriguing feature of this system is that although distant nests are genetically differentiated, workers do not reject conspecific workers from nests of the same population, and workers move freely between neighbouring nests (Chapuisat et al., 2005; Holzer et al., 2006). However, this absence of aggression is not due to a complete lack of recognition. Workers are able to distinguish nestmates from non-nestmates, as shown by higher trophallaxis rate and longer antennation bouts between non-nestmates (Chapuisat et al., 2005; Holzer et al., 2006). Together, these new data raise the question whether workers might be able to discriminate and reject foreign queens.

The main aim of this study was to examine whether resident workers reject young foreign queens that enter into their nest. We used mated queens in order to simulate the natural situation where queens seek adoption in established colonies after the mating flight. We introduced mated queens into their natal nest, a foreign female-producing nest, or a foreign male-producing nest, and measured their survival after 22 hours. This experiment also permits to test one element of the queen replenishment hypothesis, which is that queens should be equally accepted in their natal nest and in foreign female-producing nests, but rejected in male-producing nests. In addition, we also introduced young virgin and mated queens into their natal nest to examine whether the mating status of the queens influences their survival and acceptance by workers.

Material and methods

The experiment was carried out within two populations of *F. paralugubris* in Bois de Peney and Chalet à Roch in the Swiss Jura Mountains. The introductions took place during the mating flight between June 8th and 18th, 2005. We collected newly emerged alate queens and males from the nest surface between 9:15 and 10:35 am. The queens from each nest were divided in two groups, one with queens destined to mate and the other with queens destined to remain virgin. Each group was placed in a transparent plastic box (23 x 39 x 15 cm) covered with a thin tissue. Males were added and were left with the queens for approximately two hours in one of the boxes, with a ratio of queens to males of approximately 1 : 1.3. Virgin and mated queens received the same treatment with the only difference that virgin queens had no contact with males.

Queens were inserted into the nests on the same day. They were placed individually into 7 to 8 cm long (diameter: 1.5–2 cm) bamboo tubes with 2 mm diameter holes. The holes were big enough to allow workers to freely enter and leave the tubes, while trapping the larger

queen inside the tube (Fortelius et al., 1993). A previous study in *Formica exsecta* has shown that this method is effective to reveal variation among nests in the degree of queen acceptance by workers. Indeed, among populations, the mortality of queens introduced into male-producing nests ranged from 73% to 98%, in sharp contrast to the mortality of queens introduced into their natal colony, which ranged from 3 to 25% (Brown et al., 2003). To avoid aggressive reactions of workers towards the queens due to the odour of the bamboo tubes, we introduced the tubes into the nests for approximately three hours prior to the start of the experiment.

In the early afternoon, we introduced tubes containing one queen into the upper part of the mound, 10 to 20 cm under the nest surface. The virgin queens were introduced into their natal nest. The mated queens were introduced into their natal nest, a foreign female-producing nest, and a foreign male-producing nest (three treatments). Each queen and each nest was used only once. In total we used 33 different nests, resulting in 11 combinations of three nests, and eight queens per nest and treatment. The tubes with the queens were collected in the morning of the next day, 22 hours after their introduction. We recorded whether the queen was alive or dead. Occasionally, we were not able to retrieve all the tubes, so the number of introduced queens per treatment was 7.48 ± 0.66 (mean \pm SD). The queens were stored in ethanol and dissected to verify their mating status, which is revealed by the presence or absence of sperm in the spermathecae.

Out of the 88 presumed virgin queens, eight turned out to be inseminated. These queens had mated within their natal nest before sampling. The mating success of queens in boxes was very high, with 249 out of 260 queens (96%) having sperm in their spermathecae. These eight mated queens and eleven virgin queens were removed prior to analysis, but we checked that transferring them into the other treatment would not have changed the results.

On June 10th and 11th 2008, we performed an additional control experiment to verify that queen mortality was due to worker behaviour and not to queen manipulation, mating or confinement *per se*. We collected newly emerged alate queens from 11 nests at Bois de Peney and let them mate in cages as described above. For each of the 11 nests, eight mated queens were placed in tubes that were introduced into a randomly selected foreign nest, whereas eight mated queens were placed in tubes that were inserted into worker-free woodpiles located on a small meadow within our study site. The tubes were left for 22 hours and mortality was recorded as described above. In addition, worker behaviour towards queens was observed shortly after retrieving the tubes from the nests, and queen injuries were recorded.

To compare the acceptance rate of mated queens in their natal nest, a foreign female-producing nest, or a male-producing nest, we computed a two-way analysis of variance (ANOVA) on the odds of survival with nest of introduction and the date of introduction as fixed factors and nest as random factor. Similarly, to compare the acceptance rate of virgin and mated queens in their natal nest, we performed a two-way ANOVA on the odds of survival with mating status and the date of introduction as fixed factors and nest as random factor. We compared the survival of mated queens in tubes introduced in nests or in woodpiles with a Wilcoxon matched-pairs signed-rank test on the proportion of surviving queens. The power analysis was performed with the software G*power (Faul et al., 2007).

Results

Mated queens introduced into their natal nest, a foreign female-producing nest, or a foreign male-producing nest did not differ significantly in their survival rate (transformation $\log+1$, $F_{2,20} = 1.10$, $P = 0.35$, Fig. 1). Specifically, the average survival rate \pm SD of young mated queens was $20\% \pm 22\%$ when introduced into their own nest, $34\% \pm 32\%$ when introduced into foreign female-producing nests, and $17\% \pm 18\%$ when introduced into

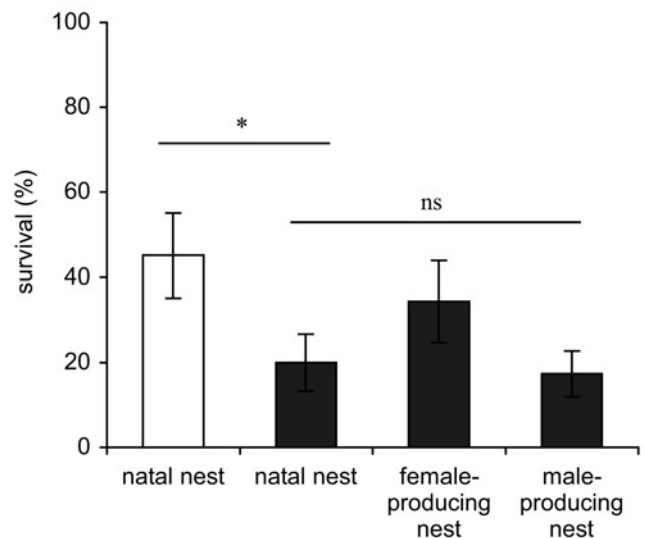


Figure 1. Survival of virgin queens introduced into their natal nest (white bar) and of mated queens (black bars) introduced into their natal nest, a foreign female-producing nest or a foreign male-producing nest. The mean \pm SE is shown.

foreign male-producing nests. The power analysis revealed that an increase of 57% in survival in one of the treatments would have been detected with a probability of 80%.

The survival of queens introduced into their natal nest was affected by their mating status. Specifically, virgin queens were significantly more likely to survive the introduction in their natal nest (mean \pm SD: $45\% \pm 33\%$) than mated queens ($20\% \pm 22\%$; $F_{1,10} = 7.63$, $P < 0.05$, Fig. 1).

The mean survival rate of virgin and mated queens introduced into their natal nest decreased significantly over time, as the flight period progressed ($F_{1,9} = 5.35$, $P < 0.05$, Fig. 2). A similar tendency was found when mated queens were introduced into foreign nests, but the effect was not significant ($F_{1,9} = 2.01$, $P = 0.19$).

Mated queens introduced into foreign nests had significantly lower survival than mated queens kept in woodpiles without workers (Wilcoxon matched-pairs signed-rank test, $W = 66$, $N = 11$, $P < 0.001$). The average survival rate \pm SD of mated queens in tubes introduced into foreign nests was $23\% (\pm 29\%)$, whereas this value was $98\% \pm 5\%$ when tubes were kept in woodpiles. This control experiment indicates that queen mortality is induced by the workers' behaviour towards them, and is not merely due to manipulation, mating or confinement. Moreover, we often observed workers attacking queens when opening the tubes retrieved from nests, and 37% of the dead queens showed severe physical injuries, with head, legs or antennae having been cut off.

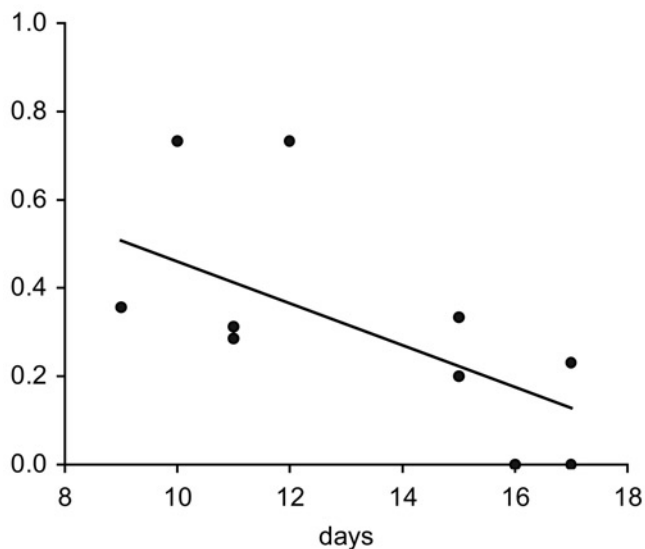


Figure 2. Mean survival of virgin and mated queens introduced into their natal nest, in relation to the progression of the mating flight period (June 8th to 18th).

Discussion

In these highly polygynous populations of *F. paralogubris*, resident workers do not discriminate against foreign queens introduced into their nest. The average survival rate of mated queens in female- and male-producing foreign nests was 25% after 22 hours, which is not significantly different from the survival rate of mated queens in their natal nest (20%). This lack of discrimination is in line with the results of previous studies of highly polygynous *Formica* ants (Rosengren et al., 1986; Fortelius et al., 1993; Sundström, 1997).

So far only one other study has investigated queen acceptance in female- and male-producing colonies of ants. In *F. exsecta* the survival rate of virgin queens ranged from 69% to 97% for introductions into natal or foreign female-producing colonies, but was as low as 2% to 27% for introductions into foreign male-producing colonies (Brown et al., 2003). In contrast, the comparatively high survival rate of mated queens in male-producing nests of *F. paralogubris* suggests that the presence of young queens within a nest is not a prerequisite for acceptance in this species, and provides no evidence for the hypothesis that workers should accept young queens only when queens are produced by the colony (Brown et al., 2003).

In our experiment the survival rate of virgin queens was twice the one of mated queens in their natal nests. This higher survival of virgin queens is in agreement with the results of previous experiments in *F. paralogubris* (Fortelius et al., 1993) and *F. truncorum* (Sundström, 1997). The most likely explanation for the higher mortality of mated queens is that workers react more aggressively towards mated than virgin queens. Workers might discriminate between the two types of queens on the basis of their cuticular hydrocarbon profiles, which are com-

monly used for recognition in ants and have been shown to differ between virgin and mated *Linepithema humile* queens (de Biseau et al., 2004). The alternative explanation that mating *per se* is costly and lowers queen survival was not supported by our control experiment, which showed high survival rate of mated queens confined in tubes in absence of workers. The short-term costs of mating have been little investigated in ants, but appear to be minimal. In the leaf-cutting ant *Atta colombica*, virgin queens and queens sampled one day after mating did not differ in their immune response, but mated queens that stored more sperm had lower immune response nine days after mating (Baer et al., 2006).

The survival rate of mated and virgin queens introduced into their natal nests was highest at the beginning of the mating season and decreased as the mating flight progressed. Again, the high survival of queens in absence of workers suggests that this rise in queen mortality is due to an increase in worker aggression over the season, possibly triggered by the absence of young queens in the nests at the end of the mating flight. However, some age- or season-related factors might also increase queen mortality independently of worker behaviour.

In conclusion, the similar survival rate of queens introduced into their natal or a foreign nest indicates that the limited gene flow between distant nests observed in our study population is not due to worker discrimination of foreign queens. The fact that workers do not preferentially kill foreign queens suggests that other mortality factors during the mating flight, coupled with dilution over distance and high rates of local recruitment (Chapuisat and Keller, 1999), are sufficient to limit effective gene flow among distant nests.

Acknowledgements

We thank G. Castella for providing the bamboo tubes, P. Buri for help in the field, and A. Reber for help with the statistical analyses. This study was supported by several grants from the Swiss National Science Foundation to LK and MC.

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