Absence of kin discrimination in a ponerine ant

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Kinship theory implies that individual social Hymenoptera should be able to identify kin. We tested kin discrimination in the polygynous ponerine ant Gnamptogenys striatula. Mate choice experiments showed that individuals did not pair according to kinship. Experiments on matriline discrimination revealed that workers did not preferentially groom, transport (after nest disturbance) or cannibalize (after starvation) larvae on the basis of kin, when both related and unrelated larvae were present. These results show the absence of kin discrimination for the criteria and experimental conditions used. The lack of kin discrimination during mate choice in G. striatula can be explained by male dispersion and female philopatry, which reduces the likelihood of mating between siblings and of kin-based mate choice reflected in incest avoidance. The lack of matriline discrimination by workers may reflect permanent intracolony mutualism or the high cost of discrimination. Finally, it appears that the absence of kin discrimination in such contexts in G. striatula is not incompatible with kinship theory and may have been secondarily selected during social evolution.

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Kin selection theory (Hamilton 1964) has provided a useful explanation for the evolution of altruism. When this theory is applied to social Hymenoptera, it allows us to understand the origin and maintenance of a sterile worker caste by invoking high genetic relatedness between the giver and the receiver of altruism (Hamilton 1972). This implies that the giver, namely a worker, is capable of discrimination between potential recipients on the basis of kin. In hymenopteran colonies, nestmates are often close genetically because they are the progeny of a unique queen. Thus, nestmate discrimination can be an indirect mechanism for kin discrimination. Kin recognition appears to be a common feature in animals (Hepper 1991). It has been evidenced recently in non-social arthropods (review in Fellowes 1998; mites: Faraji et al. 2000; Schausberger & Croft 2001). Nestmate discrimination is well known and widespread in social Hymenoptera (Getz 1991; Jaisson 1991). Such a phenomenon does not necessarily involve the assessment of relatedness, but instead familiarization or imprinting processes during the larval stage (Isingrini et al. 1985) and/or early after emergence (Jaisson 1973, 1980). Consequently, such processes should be controlled when studying kin discrimination.

As inbreeding is known to have deleterious effects (Charlesworth & Charlesworth 1987), mate choice can reveal kin discrimination. Outbreeding (i.e. incest avoidance) may result from two kinds of mechanisms. First, kin may not meet during the mating period because they mature at different times within the nest (protogyny or protandry), or because of sex ratio specialization between nests. Second, kin may encounter each other but not mate due to kin discrimination. A few experiments have demonstrated incest avoidance in social Hymenoptera (Ryan & Gamboa 1986; Ross & Carpenter 1991; Foster 1992; Keller & Passera 1993); some species are known to mate with nestmates (Buschinger 1989) but this strategy might be an adaptation to their extreme rarity (Wilson 1963).

Many social Hymenoptera are polygynous (i.e. several females reproduce simultaneously in a given colony) and/or polyandrous (i.e. several males mate with a single female and participate in the offspring), which results in the coexistence of several worker lines in one nest; such systems offer good models for the study of matriline or patriline discrimination involving precise kin discrimination. Patriline discrimination has been widely investigated in the honeybee. Early results seemed to support its existence (Getz & Smith 1983; Noonan 1986; Frumhoff & Schneider 1987; Page et al. 1989; Oldroyd et al. 1991, 1993), but later reanalysis casts doubt on that interpretation (see review by Breed et al. 1994; Kirchner & Arnold 2001). Matriline and patriline discrimination have not been confirmed for any wasp or ant species (Carlin 1989; Queller et al. 1990; Snyder 1992, 1993; Carlin et al. 1993;...
Balas & Adams 1996; Bernasconi & Keller 1996; DeHeer & Ross 1997; Heinze et al. 1997; Strassmann et al. 2000). Therefore, the absence of matriline or patriline discrimination might be a general feature in social Hymenoptera (Keller 1997).

The ponerine ant *Gnamptogenys striatula* displays both queenright and queenless colonies in nature (Blatrix & Jaisson 2000, 2001). In queenless colonies, mated workers (named gamergates by Peeters & Crewe 1984) perform sexual reproduction. Fifteen days after queen removal, some workers display sexual calling postures in the nest chambers; any males in the foraging area are carried into the nest where mating occurs (Blatrix & Jaisson 2000). This behavioural sequence, which is easy to induce in the laboratory, provides an opportunity to test for kin discrimination when males are carried into the nest and during mating. Queenright and queenless colonies are polygynous (Blatrix & Jaisson 2001). Only one or two queens produce a cohort of winged females, which are adopted in their mother colony after mating, resulting in a high genetic relatedness between nestmate queens ($b = 0.65 \pm 0.25$, Giraud et al. 2001). Males are believed to disperse (Blatrix 2000). Most queens mate only once and colonies reproduce by budding (Giraud et al. 2000).

The occurrence of multiqueen colonies allows us to test for kin discrimination during interactions between matrlines.

In this study we looked for kin discrimination via both mate choice and matriline discrimination in *G. striatula*. Different proximate mechanisms must underlie individual behaviour in these two situations, which thus provides two independent tests for kin discrimination in the same organism. We investigated kin discrimination via mate choice during transport of males by foragers and during mating in queenless colonies. We tested kin discrimination via matriline discrimination by measuring worker–larva interactions in three cases: grooming of larvae, transport of larvae after nest disturbance and cannibalism of larvae after starvation.

### MATE CHOICE

#### General Experimental Procedure

Kin discrimination during mate choice was tested in two experiments. In experiment 1, foragers from queenless nests were presented with two lines of males, one being unrelated and the other consisting of nephews; we recorded which line they transported first into the nest. In experiment 2, males were presented with two lines of workers, one unrelated and the other consisting of aunts; we assessed preferential mating by dissecting workers. We used only males produced by workers in these experiments because the queen and the workers could both produce males in monogynous queenright colonies. As a consequence we had to use nephews instead of brothers when males that were related to workers were needed.

Lines of test workers were produced as follows (Fig. 1). Twelve queens from four natural polygyrous colonies were reared separately (in the presence of 100 workers) to obtain 12 distinct lines of sister workers. Lines of test males were obtained by isolating 50 sister workers taken from each of these artificial monogynous colonies and placing them in new queenless nests. After 2 months, these workers produced males. These ‘M colonies’ provided 12 lines of males. Test workers and males were always unfamiliar even when related because males were produced by a set of isolated workers. Thus, direct familiarization could not account for potential evidence of kin discrimination.

#### Experiment 1: Transport of Males

**Methods**

One month after M colonies were set up, we isolated 100 newly emerging sister workers from eight monogynous colonies, and placed them in artificial nests. At least 1 month after the last worker joined the group (in order to allow enough workers to become foragers), we tested each of these eight experimental colonies using the following procedure: 10 marked males from an unrelated M colony and 10 marked males from the sister M colony (i.e. nephews) were simultaneously introduced in the outside area. Males transported into the nest by foragers could both produce males in monogynous queenright colonies. As a consequence we had to use nephews instead of brothers when males that were related to workers were needed.

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Results

Out of eight experimental colonies, only one preferentially transported unrelated males, whereas the remaining seven showed no significant differences (Fig. 2, a low mean rank of transport indicates a preference expressed through a faster transport). However, after treatment by the sequential Bonferroni test, no $P$ value remained significant, showing no overall preferential transportation of kin or nonkin.

Experiment 2: Mating

Methods

Six experimental queenless colonies were set up each with two unrelated lines of 26 young sister workers, obtained from two unrelated monogynous colonies (i.e. initiated by queens from different natural colonies). These workers were individually marked with a numbered label on the thorax. Unrelated workers did not fight because aggression is not seen among very young adults. Each of the six experimental colonies was replicated to obtain six pairs of experimental colonies. At least 1 month afterwards, we introduced 10 nephew males of one of the two worker lines in each experimental nest. For each pair of experimental colonies, one colony received the nephews of one worker line and the other colony received the nephews of the other worker line, in order to control a possible bias in mating ability between worker lines. Males came from M colonies. Males therefore were able to mate with aunts or unrelated workers. It is not known whether males or females perform mate choice in this species. If only females choose mates, our experimental design would show whether workers decided to mate or not. For instance, in the case of inbreeding avoidance, the line of unrelated workers would mate, whereas the aunts would refuse to do so. Thus, the distribution of mating between the two lines of workers gave information on mate choice. One month after male introduction, we dissected all surviving workers (572 out of 624, for the 12 experimental colonies altogether) after freezing to detect mating. We calculated the percentage of mated workers for each category of workers (aunts or nonrelatives of the introduced males) in each experimental colony.

Results

Table 1 gives the number of virgin and mated workers and the percentage of mated workers for each category (aunt of, or unrelated to the introduced males) for each of the 12 experimental colonies. No significant difference was found between the percentages of inseminated aunts

![Figure 2. Choice between nephews and unrelated males transported into the nest by foragers of experimental queenless colonies. The $P$ level refers to the two bars directly below it. The error bars represent standard errors.](image)
and unrelated workers (X ± SD: mated aunts: 17.8 ± 10.6%; mated unrelated: 18.9 ± 9.4%; Wilcoxon matched-pairs signed-ranks test: T=29, N=12, P=0.73).

TEST FOR MATRILINE DISCRIMINATION

General Experimental Procedure

Matriline discrimination was tested through three experiments. Workers were presented simultaneously with unfamiliar sister and nonsister larvae. We attempted to determine which line of larvae was treated preferentially by counting groomings (standard condition, experiment 1), by recording which line the workers carried back into the nest first when all the colony was placed in the outside arena (stress condition, experiment 2), and by recording which line was more cannibalized during starvation (stress condition, experiment 3). Nonsister larvae were produced two ways. First, they were produced by a nestmate queen (i.e. a queen present in the original nest of the workers but not their mother and not present in their current nest) in order to fit as well as possible the natural situation (experimental colonies of type A). Second, they were produced by an unrelated non-nestmate queen (i.e. a queen from another original colony) in order to increase the genetic difference between the two lines of larvae and, thus, to make the potential choice easier for the workers (experimental colonies of type B). Given the absence of universal cues allowing matriline discrimination, phenotype matching (as defined by Holmes & Sherman 1983) is the only plausible mechanism to perform such a task. If the workers used self-matching to discriminate between matrilines within their colony, they were expected to discriminate between colonies as well.

We used only worker-destined larvae in the experiments. Determining which larvae will be reared as reproductives would have been the best test for matriline discrimination but the production of winged queens is a rare event in the laboratory, preventing us from performing such a test. Lines of workers and larvae were obtained by rearing individual queens in the presence of 100 workers. Experimental colonies were set up with one line of workers and two lines of larvae, one being sisters of the workers and the other one nonsisters. In experiment 1, the setup of experimental colonies of type A was slightly modified. In this case, each experimental colony contained two lines of workers produced by nestmate queens and the larvae from both lineages, allowing us to test two lines of workers simultaneously. Some experimental colonies were used for several experiments. Worker and larvae lines contained 28–62 and 8–26 individuals, respectively. Workers and nonsister larvae were unfamiliar, having been reared in separate nests by different queens. Workers and sister larvae taken from the same monogygous colony were unfamiliar because workers were isolated 25 days before larvae were added. Thus, direct familiarization could not account for potential evidence of kin discrimination. In each experimental colony, one line of larvae (either the sister or nonsister line) was coloured red by providing food dyed with Fat Red B (Sigma, Lyon, France) for 10 days. Only large larvae were used because they accumulated a large amount of dye, which made observation easier. Larvae beginning to weave cocoons in experimental colonies were systematically replaced. In experiment 1, workers were marked individually with a numbered label on the thorax.

Experiment 1: Grooming of Larvae

Methods

We set up four experimental colonies of type A (i.e. eight lines of workers) and four of type B from eight polygynous natural colonies. We scanned each experimental nest every 2 min for 14–40 h. A scan consisted in recording, for each larva groomed, the identity of the worker and the colour of the larva. We calculated two indices for each worker: (1) the number of groomings given to sister larvae divided by the number of larvae in this line and (2) the number of groomings given to nonsister larvae divided by the number of larvae in the other line.

Results

In only one of eight experimental lines of type A, workers displayed significantly more groomings towards their sister larvae than towards the nestmate nonsister larvae (Fig. 3). However, the sequential Bonferroni procedure gave no significant P value, showing that workers did not preferentially groom sister larvae.

In only one out of four experimental lines of type B, workers displayed significantly more groomings towards their sister larvae than towards non-nestmate (unrelated) larvae (Fig. 3). This difference remained significant after applying the sequential Bonferroni test.
sister larvae preferentially. Nestmates (experimental colonies of type B; Wilcoxon matched-pairs signed-ranks test), either when nonsister larvae were between these ranks for any colony (Wilcoxon matched-pairs test specifically for kin discrimination based on self-avoided familiarization between actors, allowing us to note easily the order in which the larvae of the two lines were transported. For each test, we calculated the mean rank of transport for each of the two lines of larvae.

Methods

We set up five experimental colonies of type A and four of type B from eight polygynous natural colonies. We tested each experimental colony 10 times using the following procedure: workers and larvae were exposed to direct light in a transparent plastic box that opened on a 25-cm-long trail leading to the nest. The trail was made with 15 μl of an extract obtained by crushing foragers in pentane. The trail allowed workers to transport larvae quickly into the nest and, thus, allowed the observer to determine the order in which the larvae of the two lines were transported. For each test, we calculated the mean rank of transport for each of the two lines of larvae.

Results

Figure 4 gives the mean rank of transport between sister and nonsister larvae over the 10 tests applied to each experimental colony. No significant difference was found between these ranks for any colony (Wilcoxon matched-pairs signed-ranks test: \( T=16, N=10, P=0.24; T=15.5, N=10, P=0.22; T=24, N=10, P=0.72; T=27, N=10, P=0.96; T=18, N=10, P=0.33 \)) or non-nestmates (experimental colonies of type B: \( T=18, N=10, P=0.33; T=24, N=10, P=0.72; T=20, N=10, P=0.44; T=9, N=10, P=0.21 \)), showing that workers did not transport sister larvae preferentially.

Experiment 3: Cannibalism of Larvae

Methods

Cannibalism of larvae occurs in G. striatula colonies (personal observation). Workers always initiate cannibalism, but both larvae and workers can feed on the cannibalized individual. We set up four experimental colonies of type A and four of type B from eight polygynous natural colonies. After a 1 week of starvation, we calculated the proportion of surviving larvae for each line.

Results

The proportion of surviving larvae did not differ significantly between sister larvae and nestmate larvae (Table 2, experimental colonies of type A; Wilcoxon matched-pairs signed-ranks test: \( T=3, N=4, P=0.47 \)). The same result was observed between sister larvae and larvae from another colony (Table 2, experimental colonies of type B; \( T=5, N=4, P=1 \)), indicating that workers did not preferentially initiate cannibalism on nestmate (nonsister) or non-nestmate larvae compared to sisters.

The results in Table 2 allowed us to calculate that 67% of noncoloured larvae survived versus 48% of red-dyed larvae. This difference might indicate a slight effect of dying on survivorship. In the above experiments, sister larvae were alternatively dyed red or not dyed, so dying per se could not bias our results.

DISCUSSION

Choice experiments showed that foragers from queenless colonies did not transport males into the nest according to relatedness. Moreover, relatedness did not influence pairing of males and mating workers. These results suggest the lack of mate choice based on kinship. Tests for matriline discrimination showed no differential treatment between related and unrelated larvae by workers, both under normal conditions (experiment 1) and under harsh conditions (experiments 2 and 3). The fact that discrimination did not occur even when unrelated larvae were not nestmates demonstrates that workers do not display matriline discrimination in normal colonies. The experimental procedure used in the present study avoided familiarization between actors, allowing us to test specifically for kin discrimination based on self-matching and/or cues from the discriminated individual.
Hamilton’s rule (Hamilton 1972) states that an altruistic behaviour from an individual x towards an individual y can be selected for and maintained only if the following condition is fulfilled: \( C_x \times b_{xy} < B_y \), where \( C_x \) is the cost of altruism for x, \( b_{xy} \) is the relatedness between x and y, and \( B_y \) is the benefit of altruism for y. In polygynous and/or polyandrous colonies, \( b_{xy} \) is lower than in monogynous and/or monandrous colonies. In the absence of matriline and/or patriline discrimination, \( b_{xy} \) remains low on average, and so \( B_y \) is expected to be high in order to compensate for \( b_{xy} \) and fulfill the condition \( C_x < B_y \). Thus, the absence of matriline and/or patriline discrimination suggests that polygyny and polyandry were selected and maintained because of their advantages, independent of the implication of a kin discrimination process. In G. striatula, one of these advantages could be the coexistence of genetically diverse matriline, leading to a more efficient division of labour (Blatrix et al. 2000).

Results on both mate choice and matriline discrimination indicated that G. striatula did not display kin discrimination in these two contexts. This might be explained either by the lack of recognition capacity, or by the absence of a visible discrimination behaviour in workers and males. The fact that workers groomed sister larvae preferentially in only one case (experiment 1) suggests that absence of kin discrimination does not necessarily mean the absence of kin recognition. The absence of a visible discrimination behaviour could be explained either by the decision rules of the ants, or by the inability of the experimental design and statistical analysis to detect such behaviour. In particular, statistical hypothesis testing is prone to errors in detecting differences (Johnson 1999); failing to reject the null hypothesis (this study) does not mean that it is true. Behavioural studies on mate choice and matriline discrimination in social insects are in their early stages and more data are needed to draw a general pattern, if any. In such studies, it is of the greatest importance to consider nonsignificant results with the same interest as significant ones, despite the bias stated above, because they can indicate biological strategies improving our understanding of behavioural evolution. Thus, there is a need for new statistical approaches better adapted to such data.

Although our results showed the absence of kin discrimination in two particular contexts (mate choice and matriline discrimination), this study does not rule out kin discrimination in G. striatula. In fact, it could occur in many other contexts not tested here. But the fact that kin discrimination was not used in the contexts we tested may shed light on other evolutionary strategies occurring in these ants. These strategies are discussed below.

Giraud et al. (2000) demonstrated that the average relatedness between mates of G. striatula does not differ significantly from zero. Although this was calculated for queens and not for gamergates, the situation is probably similar because queenright colonies reproduce by budding and winged females apparently do not swarm (Giraud et al. 2000). Male dispersion might account for the absence of mate choice. In the case of G. striatula, siblings have little chance to breed because of male dispersion and female philopatry, so there is little chance for incest avoidance to evolve. Because of their haplodiploid system of sex determination, Hymenoptera are expected to be particularly sensitive to inbreeding. In fact, inbreeding results in the production of diploid males (sterile), which constitute a load for the colony. This load could prevent colonies from succeeding (Ross & Fletcher 1986). However, experimental inbreeding did not show any deleterious effects in some ants and other social Hymenoptera (Haskins & Whelden 1965; Mintzer 1982; Clarke et al. 1986; Keller & Passera 1993; Gerloff et al. 1998). Two characteristics of social Hymenoptera might be responsible for their high resistance to the deleterious consequences of inbreeding: (1) male haploidy has been proposed to result in selection against recessive deleterious alleles, and thus, in a decrease of their frequencies (Askev 1968); and (2) brood cannibalism might allow energy recycling from nonviable, inbred individuals (Keller & Passera 1993). These may constitute an alternative explanation for the lack of mate choice.

Matriline discrimination seems not to be the rule in G. striatula. However, in one case more groomings to sister larvae were detected. This is probably not due to chance because this case remained significant even after Bonferroni correction, suggesting that the capacity to recognize kin does exist. In honeybees, cuticular hydrocarbons differ between patrilines (Arnold et al. 1996). Moreover, kin discrimination seems to be influenced by genetic cues in at least two species of social Hymenoptera (Greenberg 1979; Keller & Passera 1993). Thus, even if more studies on the genetic component of kin discrimination are needed, it appears that all the prerequisite conditions for matriline and/or patriline discrimination do potentially exist. Several hypotheses have been suggested to explain why matriline and/or patriline discrimination is not the rule in social insects (Keller 1997). If each individual can choose her interaction partner, this might result in a decrease in colony efficiency (Ratnieks & Reeve 1992), particularly in colonies with a high number of worker lines (for example in honeybee and highly polygynous ant species). Moreover, the cost of errors made by the recognition system may exceed the benefit of discrimination (Reeve 1989). Thus, cooperation between all individuals might constitute a better adaptation than discrimination.

Kin discrimination has been confirmed for many social insects. In some social insects, however, such as G. striatula, it may be absent in particular contexts. In this species, the interpretation of this trait (analysing the life history cycle and the evolutionary implications of matriline discrimination) leads us to argue that it is consistent with kinship theory. Thus, the absence of kin discrimination may constitute a further step in social evolution.

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