Sham nepotism as a result of intrinsic differences in brood viability in ants

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In animal societies, cooperation for the common wealth and latent conflicts due to the selfish interests of individuals are in delicate balance. In many ant species, colonies contain multiple breeders and workers interact with nestmates of varying degrees of relatedness. Therefore, workers could increase their inclusive fitness by preferentially caring for their closest relatives, yet evidence for nepotism in insect societies remains scarce and controversial. We experimentally demonstrate that workers of the ant *Formica exsecta* do not discriminate between highly related and unrelated brood, but that brood viability differs between queens. We further show that differences in brood viability are sufficient to explain a relatedness pattern that has previously been interpreted as evidence for nepotism. Hence, our findings support the view that nepotism remains elusive in social insects and emphasize the need for further controlled experiments.

Keywords: nepotism; egg viability; kin recognition; social insects; ants

1. INTRODUCTION

Individuals can pass on their genes not only directly through their own offspring, but also indirectly by favouring the reproduction of relatives such as siblings or cousins (Hamilton 1963, 1964). Hamilton's principle of kin selection is a powerful explanation for the evolution of reproductive altruism and cooperation in groups with related individuals. However, conflicts can arise when individuals have divergent genetic interests.

A prominent conflict predicted by kin selection is nepotism, a behaviour whereby individuals favour their closest relatives over less-related individuals (Hamilton 1987). In social insect colonies with more than one mother or father (Bourke & Franks 1995; Crozier & Pamilo 1996) workers could increase their inclusive fitness by favouring individuals belonging to the same matriline or patriline as long as the costs of discrimination are low (Ratnieks & Reeve 1991).

The decision of an individual to behave nepotistically depends on the ability to correctly identify the relatedness of the recipient and thus on the types of cues available (Reeve 1989; Breed 1998). The chemical recognition labels in social insects are a combination of genetically specified and environmentally acquired cues (Gamboa *et al.* 1986; Crozier & Pamilo 1996; Downs & Ratnieks 1999). Although cuticular cues are individualistic, feeding and grooming homogenize the recognition labels among nestmates (Gamboa *et al.* 1986; Soroker *et al.* 1995; Arnold *et al.* 2000; Boulay *et al.* 2000; Lenoir *et al.* 2001). The masking or active scrambling of recognition cues may prevent nepotism (Keller 1997).

Early claims of widespread nepotism in honeybees (Getz & Smith 1983; Page *et al.* 1989) have been dismissed on statistical or biological grounds (Oldroyd *et al.* 1990; Frumhoff 1991; Breed *et al.* 1994). Several studies in the honeybee (*Apis mellifera*; reviewed by Tarpy *et al.* 2004; see also Châline *et al.* 2005) in wasps (Queller *et al.* 1990; Strassmann *et al.* 1997; Solis *et al.* 1998; Strassmann *et al.* 2000) and in ant species with multiple-queen colonies (Carlin *et al.* 1993; Snyder 1993; Balas & Adams 1996; Bernasconi & Keller 1996; DeHeer & Ross 1997) failed to detect nepotism. Thus, the bulk of the evidence suggests that social insects do not behave nepotistically, probably because of the costs entailed by decreased colony efficiency or discrimination errors (Keller 1997; Ratnieks *et al.* 2006). Recently, the consensus that nepotistic behaviour does not occur in insect colonies was challenged by a study in the ant *Formica fusca* (Hannonen & Sundström 2003) showing that the reproductive share of queens more closely related to workers increases during brood development. However, this pattern can be explained either by nepotism with workers preferentially rearing the brood of more closely related queens or intrinsic differences in the viability of eggs laid by queens.

We designed an experiment to disentangle nepotism and differences in brood viability. We used the ant *Formica exsecta*, a species in which colonies can contain several queens (Brown & Keller 2000). We tested if workers prefer to rear their kin when given the choice between highly related and unrelated brood. We also looked for differences in egg viability among queens and simulated if such differences in egg viability may mistakenly lead to the conclusion that workers behave nepotistically.

2. MATERIAL AND METHODS

(a) *Field collection*

We collected *F. exsecta* ants from polygynous (multiple-queen) colonies at La Dunanche (46°28′ N, 6°11′ E) in the Swiss Jura Mountains between 21 and 25 April 2004. From each of 104 colonies, we sampled one queen and approximately 500 workers. These colonies were kept in nests (26×41×15 cm) containing peat as nest material and a tile as nesting site at 22–24 °C for two weeks. Ants were regularly fed with honey water and a mixture of agar, egg, honey and water.

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(b) Experimental set-up

To create experimental colonies consisting of sister workers and to control for environmental recognition cues, we reared workers apart form the maternal colony. We transferred brood (mean $\pm$ s.d., 186 ± 87) from the single-queen colonies to nests containing approximately 50 colour-marked workers originating from a mix of workers from all experimental colonies previously collected in the field. Since colonies were sampled randomly from the population the workers in this mixed stock were unrelated. The colour-marked workers were removed from the colonies once the transferred brood hatched from the pupae. Overall, we obtained sufficient numbers of sister workers (mean $\pm$ s.d., 55 ± 31) in 50 colonies. These colonies were arranged in pairs comprising equal number of workers. Colonies within a pair were each provided with equal number of eggs and small larvae collected from the two colonies containing the mother queens of the workers (mean $\pm$ s.d., 61 ± 44 brood items per colony). The 25 experimental colony pairs were fed twice a week with a number of fruit flies equal to half of the number of workers and eggs. Water and honey water were provided *ad libitum*. Nineteen of the 25 colony pairs produced five or more pupae that were collected and prepared for genetic analyses.

(c) Genotyping and maternity assignment

To assign maternity of the reared pupae to one of the two queens, we genotyped all pupae and six workers per colony at four highly polymorphic microsatellite loci; FL21 (Chapuisat 1996), FE13, FE17 and FE37 (Gyllenstrand *et al.* 2002). DNA was extracted from the head of workers or the entire pupae in 250 and 500 µl of 5% Chelex, respectively. Samples were incubated for 20 min at 90 °C. The loci FE13 and FL21 were amplified in a simplex PCR and the loci FE17 and FE37 were co-amplified in a multiplex PCR (Kümmerli *et al.* 2005). PCR products were mixed and run on an automatic sequencer (ABI Prism 377XL). The number of alleles per locus ranged from 6 to 19 (mean $\pm$ s.d., 12.5 ± 5.3) with an expected heterozygosity between 0.59 and 0.88. We used the genotypes of the workers within a colony to determine the genotypes of the queens and their mates. Maternity of all pupae could be determined without ambiguity because the two queens within colony pairs were unrelated.

(d) Statistical analyses

The relatedness among sister workers was calculated with the program RELATEDNESS v. 5.0.8 (Queller & Goodnight 1989). We further estimated the relatedness of sister workers towards the reared offspring from the mother or the foreign queen, respectively. To test whether the observed proportion of sisters reared was significantly greater than 0.5, the expected ratio in absence of nepotism, we performed a one-sample *t*-test (one tailed) on the weighted mean proportion of sisters reared across all colony pairs (Sokal & Rohlf 1995). The power of the *t*-test was calculated with the computer program G-POWER (Erdfelder *et al.* 1996) by increasing the mean ratio of nepotism in steps of 0.01 starting at 0.5. The power was calculated for each step until it reached 80%.

To test for differences in brood viability between pairs of queens, we counted the number of cases where one queen contributed more to the reared offspring in both colonies in the pair and compared it to the expected frequency of 0.5 with a binomial test.

We used the procedure described by Hannonen & Sundström (2003) to compare the queen's increase in reproductive share between the egg and adult stages and her relative relatedness (relatedness index) towards the workers they would produce given their observed difference in brood viability. Since the relatedness was measured over few loci, stochastic errors can result in negative relatedness indices.

3. RESULTS

The relatedness (mean $\pm$ s.e.m.) between the sister workers was $r = 0.67 \pm 0.02$. The relatedness value is lower than expected for full sisters ($r = 0.75$), because 34% of the queens had mated with multiple males (mean mating frequency $= 1.4$). Moreover, the relatedness between workers and the reared brood from the mother was $r = 0.68 \pm 0.03$, this was significantly higher than the relatedness between workers and the reared brood from the unrelated queen which was only $r = 0.04 \pm 0.06$ (paired *t*-test: $t_{33} = 15.3$, $p < 0.001$). The relatedness confirmed our experimental set-up: sister workers were given the choice to rear highly related or unrelated brood.

Among the 342 offspring genotyped, the proportion of sisters was 0.52 ± 0.02 (weighted mean $\pm$ s.e.m.), which is not significantly greater than the expected ratio of 0.5 in the absence of nepotism (one-sample *t*-test (one-tailed): $t_{18} = 1.05$, $p = 0.31$). A power analysis shows that a degree of nepotism of 0.56 (proportion of sisters reared) would have been detected with a probability higher than 80%.

Our paired design allowed us to compare the viability of the brood between the paired queens. In 16 (84.2%) out of the 19 queen pairs, the same queen had a higher proportion of brood reared to adulthood, regardless of whether the brood was reared by her daughters or the workers of the other queen (binominal test with $P_{Ho} = 0.5$: $n = 19$, $p = 0.004$). While this further supports the view that workers fail to discriminate between kin classes, the data also indicates that queens differ in the viability of their brood.

We further found a significant positive correlation between a queen's increase in reproductive share during brood development and her relative relatedness towards the workers that were reared (Pearson's correlation coefficient: $r_p = 0.65$, $n = 19$, $p = 0.003$), a pattern caused by intrinsic difference in egg viability between queens and not worker nepotism (figure 1).

4. DISCUSSION

The main result of this study is that workers of the ant *F. exsecta* did not behave nepotistically when they had the choice to rear sisters or unrelated individuals in a laboratory experiment. We controlled for environmental recognition cues by rearing the sister workers apart from their maternal colony. Moreover, in a natural colony of ants with multiple breeders, workers will usually interact with multiple kin classes of several degrees of relatedness. Our experimental set-up with two kin classes in a homogenous environment therefore should have facilitated nepotistic behaviour.

Studies supporting nepotism in social insects are rare or controversial (Keller 1997; Ratnieks *et al.* 2006). In honeybees, no clear evidence for nepotism was detected (reviewed by Breed *et al.* 1994; Tarpy *et al.* 2004). Several studies in wasps found no support for within colony kin discrimination (Queller *et al.* 1990; Strassmann *et al.* 1997; Solis *et al.* 1998). In the fire ant *Solenopsis invicta*
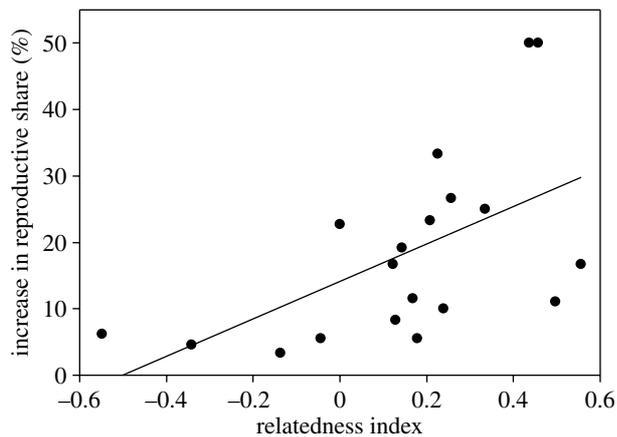
Figure 1. Sham nepotism in the ant *Formica exsecta*. The significant positive correlation between a queen's increase in reproductive share and her relative relatedness towards workers is caused by intrinsic difference in egg viability between queens and not worker nepotism. The relatedness index was calculated as $r_{wq^+} - r_{wq^-}$, where r_{wq^+} is the relatedness of workers to the queen that increased her reproductive share and r_{wq^-} is the relatedness of workers to the queen whose share decreased.

workers did not favour their mother during fights between co-foundress queens (Balas & Adams 1996; Bernasconi & Keller 1996), nor did they tend or feed preferentially the more related queen in multiple-queen colonies (DeHeer & Ross 1997).

A proximate explanation for the lack of nepotism in *F. exsecta* and other social insects might be that the variability of the genetic recognition cues is not sufficient to correctly identify and discriminate between related and unrelated brood. In the ant *Formica truncorum*, information based on genetic cues is limited, which may constrain the kin assessment that is necessary for nepotism (Boomsma *et al.* 2003). Similar results were found in the wasp *Vespa crabro* where the differences in the chemical labels between patrilines were low (Dani *et al.* 2004).

At the ultimate level, the cost of nepotistic behaviour may outweigh the benefits. In social insect colonies with multiple kin classes such as *F. exsecta*, assessing relatedness differences on the basis of genetic cues might be difficult and error prone. Moreover, nepotistic behaviour is likely to decrease colony efficiency. This may result in a net decrease in inclusive fitness for all colony members, so that nepotistic behaviour is selected against (Keller 1997).

In contrast to the view that nepotism is absent in social insects, a recent study in the ant *F. fusca* found that the reproductive share of the queens more related to the workers increased during brood development (Hannonen & Sundström 2003). This correlation was interpreted as nepotism, with workers preferentially rearing the brood of more closely related queens. However, this result can also be explained by intrinsic differences in the viability of eggs laid by queens. If brood viability differs among queens, the majority of adult workers in a colony will be the daughters of the queen whose eggs have the higher likelihood of developing to adulthood. As a result, workers will collectively be more related to this queen. Empirical data on intrinsic brood viability are thus essential to interpret patterns of apparent nepotism based on changes in matrilines frequency during brood development.

The second important result of our study is indeed that *F. exsecta* queens differed in brood viability. Assuming that queens have similar fecundities, we estimated the relatedness between each queen within a pair and the workers they would produce given their observed difference in brood viability. Our analysis revealed a significant positive correlation between the difference in relatedness of the two queens towards the workers they would produce and the increase in reproductive share between the egg and adult stage (figure 1). It is likely that differences in brood viability are common in social insects (Martin *et al.* 2004). Hence, a positive association between queen–worker relatedness and differential brood survival within colonies can emerge without workers behaving nepotistically.

In conclusion, this study reveals that workers fail to discriminate between related and unrelated brood in *F. exsecta*. However, there are significant and consistent differences between queens in the viability of their brood. Our findings support the view that nepotism remains elusive in social insects (Keller 1997; Queller & Strassmann 2002; Tarpy *et al.* 2004) and emphasize the need for further experiments controlling for differences in brood viability.

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