Giving your daughters the edge: bequeathing reproductive dominance in a primitively social bee

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The fitness associated with behavioural strategies is usually estimated in terms of offspring number and size. However, in group-living animals the reproductive value of offspring may also depend on their social rank. We show here that in an allodapine bee *Exoneura robusta*, dominant mothers can behaviourally influence their daughters' reproductive rank by controlling insemination of other potential mothers. In *E. robusta*, group living is near mandatory and reproductive dominance among female nestmates is determined by order of adult emergence. Nests are single, undivided burrows and the dominant female assumes a guarding position closest to the nest entrance. We show that before the egg-laying period, subordinate females who have been absent from the nest are 'screened' by the reproductive guard upon attempted re-entry. Those who have been in contact with foreign males are less likely to be granted access back into the nest than those who have been in contact with foreign females or with no bees at all. We argue that by controlling insemination patterns of their nestmates, dominant females ensure that their own daughters eclose first and are therefore more likely to assume dominance in the next generation. This presents a situation where dominance is bequeathed to daughters by behavioural means. The ability of mothers to influence social hierarchies in subsequent generations introduces a fitness component additional to the number and size of offspring produced.

Keywords: sociality; dominance hierarchies; bees; mating behaviour

1. INTRODUCTION

Modelling reproductive division of labour within social insect colonies usually involves a reckoning of direct and indirect fitness components of colony members. Different behavioural roles in primitively social bee species are often associated with reproductive dominance and these can influence individual reproductive output and colony productivity and survival (e.g. Hogendoorn & Leys 1993; Melna & Schwarz 1994; Schwarz 1986, 1988, 1994). The primitively social xylocopine bees (tribes Manueliini, Xylocopini, Ceratinini and Allodapini) are especially relevant to studies of behavioural differentiation because of the social diversity that occurs within this subfamily. In some species, dominant females maintain a guarding position close to the nest entrance and subordinates forage (Mason 1988; Maeta *et al.* 1992; Melna & Schwarz 1994; Maeta & Sakagami 1995; Schwarz & Hogendoorn 1998), whereas in others, guards are young pre-reproductives or old, formerly reproductive females that may be protecting tenure of the nest from potential usurpers (Hogendoorn & Velthuis 1993). In general, guarding behaviour in social insects is usually regarded as a mechanism for protecting against the invasion of

predators and parasites or unrelated conspecific potential rivals to the current social hierarchy.

Behavioural castes are well documented within the xylocopine bees, but the mechanisms for caste determination are not as well understood as for eusocial apine, meliponine and halictine bees. In the latter groups, queen-worker differentiation can be related to proximally acting age- or size-related differences (e.g. Wilson 1975; Strassmann & Meyer 1983; Fletcher & Ross 1985; Hughes & Strassmann 1988), variation in larval food (Engels & Imperatriz-Fonseca 1990), a genetic basis (Kerr 1974), or hormonal factors (Hartfelder 1990).

Exoneura robusta is a primitively social allodapine bee from south-eastern Australia. (The genus *Exoneura* is currently under taxonomic revision. The species discussed here, *Exoneura robusta* (S. G. Reyes, personal communication), was previously incorrectly referred to as *E. bicolor* (see Schwarz *et al.* (1997) for a review). Throughout this report we use the revised name, *E. robusta*, although the relevant literature to which we refer cites the species as *E. bicolor*.) Colonies nest within a single, unbranched and undivided burrow in dead tree fern fronds and are univoltine. Group living appears to be near mandatory, as females who nest alone have a very low rate of brood production (Schwarz 1988, 1994). Colony development comprises two stages (founding of new nests and re-use of these nests in subsequent generations), which differ in

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social organization. New nests are cofounded by up to eight female relatives (relatedness within nests, r ca. 0.6) who are all inseminated and reproductively active (Schwarz 1987). In the following season, nests can be re-used by daughters of the original foundresses, and nest re-use may occur over several years depending on the durability of the nesting substrate (Schwarz 1986). Colonies in re-used nests also show high relatedness among female nestmates (r ca. 0.5), but unlike new nests, are characterized by well-developed reproductive skew (Schwarz 1986, 1987). Reproductive dominance in these nests is determined by the order of adult eclosion, with the first individual to eclose becoming dominant (Schwarz & Woods 1993). Differences in adult eclosion of only a day or so are sufficient to establish a hierarchy that persists for seven months or longer. Schwarz & O'Keefe (1991) found that later-eclosing females tend to take on foraging roles and have higher mortality rates than their older nestmates, whereas first-eclosing females tend to behave as guards prior to egg laying (Schwarz & Hogendoorn 1998). These observations suggest that first-eclosing females accrue both reproductive and survival benefits, but it is unknown why dominant females should adopt guarding behaviour given the potential risks posed by enemies at the nest entrance.

Schwarz & Hogendoorn (1998) hypothesized several benefits for guarding behaviour by dominant females. These include (i) the possibility of permeating fresh air entering the nest with volatile pheromones that may inhibit ovarian development of nestmates (O'Keefe & Schwarz 1990), thereby allowing the guard to maintain dominance; (ii) preventing potential usurpers from entering the nest; and (iii) controlling the movement of nestmates into and out of the nest, and so enable dominant females to control food resources, or prevent mating by subordinate females.

In this report we test the third hypothesis and examine the response of guarding females to returning nest absentees. We conclude that subordinate females who have been in contact with conspecific males while away from the nest are less likely to be granted admittance back into the nest than those who have been in contact with conspecific females or with nothing. If females who have been in contact with foreign males outside of the nest are likely to have become mated, exclusion behaviour by guarding females has the effect of increasing the likelihood that the dominant's daughters are produced and develop first and therefore become dominant in the next generation. Conditional admittance of foragers by guarding females could be regarded as behaviourally mediated bequeathment of reproductive dominance.

2. MATERIALS AND METHODS

Intact colonies of *Exoneura robusta* were collected from the Dandenong Ranges National Park (Victoria, Australia) on 19–21 March 1997, in fern fronds of the tree fern *Cyathea australis*. Fronds were placed on ice until returned to Flinders University, Adelaide, where they were stored at 12 °C until processing. After opening the nests, occupants were transferred to artificial observation nests (Schwarz & Overholt 1993) and left for a week to acclimatize outdoors under natural photoperiod before behavioural observations began.

Replicate trials involved briefly opening an observation nest and removing a subordinate female from the basal third of the nest where dominants are unlikely to occur in autumn (Schwarz & Hogendoorn 1998). This female (hence referred to as the 'forager') was then placed in a plastic vial (1.5 cm diameter $\times$ 5 cm long) with a bee from another nest for approximately 1 min, allowing substantial physical contact between the two individuals. The non-nestmate was then removed from the vial, and the vial was placed over the entrance of the forager's nest, allowing her to attempt re-entry of her nest. Upon re-entry, observations of interactions between the re-introduced female and the nest occupants (primarily the guard) were made. Our study comprised three treatment types: (i) the forager was subjected to physical contact with a foreign male ($N=31$ replicate trials spread over $n=21$ observation nests); (ii) the forager was subjected to physical contact with a foreign female ($N=28$ trials, $n=21$ nests); and (iii) a control where the forager was placed in a vial alone ($N=33$ trials, $n=24$ nests). For each treatment, some observation nests were used more than once; in such cases the forager was selected haphazardly from the group of subordinate nestmates and the foreign bees which the foragers were subjected to were always selected from different nests, so that each trial comprised a unique forager/foreign bee combination. The person observing interactions after re-introduction of the forager was unaware which treatment the forager had been subjected to, so that data collection was blind.

Trials ran for a maximum of 10 min from the time when the forager first re-entered the nest. Trials ended earlier if the forager gained access to the nest by passing the guard. If no access occurred after 10 min, the trial was stopped.

The frequency of 12 key behaviours of guarding females and foragers was recorded. Behaviours observed involving direct interactions between the guard and the forager were as follows.

1. Guard direction reversal followed by the guard retreating (hereby referred to as 'guard reversal retreat', GRR). This most often followed antennal contact with the forager.
2. Guard direction reversal followed by the guard maintaining a blocking position with her metasoma ('guard reversal block', GRB). This behaviour limits further access into the nest by the forager.
3. Guard direction reversal followed by the guard physically nudging the forager with her metasoma toward the nest entrance ('guard reversal nudge', GRN). This behaviour effectively pushes the forager towards the nest entrance.
4. Guard reversal in nest followed by the guard pushing the forager backwards towards the nest entrance with her metasoma ('guard reversal push', GRP). This behaviour also serves to push the forager towards the entrance, but at a more continual rate than a GRN.
5. Guard blocks forager from moving further down into the nest by maintaining position with her metasoma outward ('guard block', GB). This differs from GRB because in this case, the guard is already facing with her metasoma outward.
6. Guard moves quickly forward up the nest to meet head on with 'forager' ('guard forward charge', GFC).

The remaining six recorded behaviours either did not involve the guard or did not involve interaction between the guard and the forager.

7. Guard retreats down the nest at the approach of the forager ('guard retreat', GR).

Figure 1. Histogram of mean lengths of the three largest oocytes for guarding and non-guarding individuals. Category 1 = 0–3 micrometer eyepiece units, 2 = 3.01–6 micrometer eyepiece units, 3 = 6.01–9, category 10 = 27.01–30 micrometer eyepiece units.

8. Guard preens, usually after contact with the forager and while the forager is exerting slight pressure on the guard ('guard preens', GP).
9. Forager moves quickly forward down the nest to meet head on with the guard ('forager forward charge', FFC).
10. Forager retreats back up the nest at the approach of the guard ('forager retreat', FR).
11. Forager passes a 'worker' female (i.e. not the guard) who has moved above the guard in the nest ('forager passes worker', FPW).
12. Forager preens, usually while pressure from the guard was at aimum, and not necessarily after contact with the guard ('forager preens', FP).

Two other key events were recorded for each trial: whether the forager passed the guard and re-entered the nest, or whether the forager did not gain access into the nest by passing the guard within 10 min. If the forager had not passed the guard after 10 min, this usually signified that the forager was no longer trying to pass the guard and both parties were inactive, or the forager had been completely expelled from the nest.

A numerical value (the 'guard aggressive index') was calculated by adding the frequencies of GRR, GRB, GRN, GRP, GFC and GB in each trial. An additional one point was added to the guard aggressive index for each trial if there was no successful entry by the forager after 10 min, as this indicated successful 'defence' by the guard.

Once trials were completed, individuals were fixed in Kahle's solution and the forager, the guard and a random subsample of remaining occupants were dissected in 70% ethanol to examine ovarian development. Dissections were carried out blind to status of the individual, and ovarian development was measured as the mean length of the three largest oocytes present within the ovaries (Schwarz 1986).

3. RESULTS

(a) Reproductive dominance

Schwarz & Hogendoorn (1998) showed that guarding females in colonies of *E. robusta* are usually reproductively dominant. Our results support this notion. Figure 1 shows

Figure 2. The mean ($\pm$ s.e.) guard aggressive index for the three treatments. N = the number of replicate trials. Treatment 1, forager contact with foreign male; treatment 2, forager contact with foreign female; treatment 3, forager contact with no bees.

the mean ovary sizes of guarding and non-guarding individuals used in this study. Ovary sizes of guarding and non-guarding bees were heteroscedastic (Cochran's C -test: $C_{5,15} = 0.252$, $p = 0.013$) and therefore were $\log_{10}(x+40)$ -transformed to produce homogeneity of variances ($C_{5,15} = 0.203$, $p = 0.92$). The transformed variable was subjected to two-way ANOVA where nest and individual status (guard or non-guard) were crossed factors. This revealed no effect of nest ($F_{14,71} = 1.45$, $p = 0.154$), a highly significant effect of individual status ($F_{1,71} = 33.85$, $p < 0.001$), and no interaction between the two ($F_{14,71} = 1.64$, $p = 0.091$). Guarding individuals were generally the reproductive dominants within a nest.

(b) Behavioural interactions

The guard aggressive indices are summarized in figure 2. Treatment 1 (foragers subjected to foreign males) had a mean guard aggressive index of 4.26, treatment 2 (foragers subjected to foreign females) had a mean guard aggressive index of 1.07, and treatment 3 (foragers not subjected to any other individual) had a mean guard aggressive index of 0.58. A Kruskal–Wallis one-way ANOVA revealed a significant treatment effect ($H_2 = 28.8$, $p < 0.001$). To determine which treatments differed from each other we carried out the *post hoc* multiple comparisons method for Kruskal–Wallis tests outlined by Siegel & Castellan (1988). This indicated that treatment 1 was significantly different from both treatments 2 and 3, but that treatments 2 and 3 were not different from each other. These results indicate that guards act more aggressively toward females who have been in contact with foreign males than to those that have been in contact with foreign females or no bees at all.

Figure 3 shows the proportion of trials in each treatment where no entry by the forager was gained to the nest after 10 min. Dependence of entry rates on treatment

Figure 3. The proportion of replicate trials where no entry was gained into the nest by the forager after 10 min for the three different treatments. n = the number of replicate trials. Treatment 1, forager contact with foreign male; treatment 2, forager contact with foreign female; treatment 3, forager contact with no bees.

type was assessed using a χ^2 test. This showed a significant treatment effect ($\chi^2_2 = 25.05$, $p < 0.001$). To determine where the differences leading to this result occurred, we partitioned the contingency table into two subgroups, which were then subjected to the modified χ^2 procedure outlined by Siegel & Castellan (1988). Analysis of treatments 2 and 3 showed no significant effect ($\chi^2_1 = 0.53$, $p > 0.3$). However, comparison of treatment 1 with treatments (2+3) showed a highly significant effect ($\chi^2_1 = 25.53$, $p < 0.001$). These analyses indicate that guarding females are less likely to re-admit potentially mated females back into the nest, but that re-admission rates do not differ between foragers exposed to foreign females or exposed to no bees at all. It should be noted that no individuals in treatment 1 were observed to mate during the trials.

4. DISCUSSION

The fitness consequences for controlling entry to a nest are usually considered in terms of the resources directly appropriated by the controller from nestmates, or in terms of the number of offspring produced as a result of deterring enemies. However, in group-living species with social hierarchies, the fitness of individuals may not be a simple function of colony survival or the number of offspring produced but, rather, it may depend on how these offspring interact with other members of their cohort and the characteristics of these cohorts. For social insects, there has been little research into how parents can affect the fitness of their offspring other than by controlling the resources invested in those offspring.

For *E. robusta*, nest re-use over consecutive generations and the fact that reproductive dominance in re-used nests is determined by order of adult eclosion (Schwarz & Woods 1993) creates opportunities for females of one generation to influence the social rank of their female

offspring by controlling the order in which those offspring eclose among their cohort. Dominant females begin laying eggs several weeks (during winter) before secondary egg-layers begin mating (early spring) (Schwarz 1986). This headstart increases the chance that daughters produced by the dominant will complete their development before those of the secondary reproductives. Although dominant females in naturally occurring winter nests are nearly always mated, experiments have shown that insemination is not necessary for first-eclosed females to assume and maintain dominance (Schwarz & Woods 1993), and typical dominance hierarchies have been found in naturally occurring nests where all nestmates are uninseminated (Schwarz 1986, unpublished data). Furthermore, manipulation of adult eclosion order (Schwarz & Woods 1993) and analysis of observation colonies (Schwarz & O'Keefe 1991) have shown that body size is not a determinant of reproductive dominance and that females are totipotent.

In *E. robusta*, some subordinate females become mated in spring and lay eggs additional to those produced by the dominant (Schwarz 1986). By controlling insemination status of nestmates before the winter egg-laying period, guards can ensure that if subordinates were to produce eggs over winter, these eggs would be males and not compete with the guard's daughters for dominance in the following generation. Consequently, guarding females in *E. robusta* are able to bequeath reproductive dominance to their own daughters by controlling the timing of their nestmates' mating.

However, this scenario becomes problematic when one considers why males would allow contact labels to be transferred during mating if such labels make it more difficult for females to re-enter their nests, given the costs for solitary nesting females? We cannot definitely answer this question at present but it seems likely that transferral of an 'already inseminated' label to females during mating may serve to discourage subsequent matings and thus sperm competition. Alternatively, male scent may be a strong intersexual selective agent, where males not exhibiting a scent are not attractive to mates or are not recognized as conspecifics by females. For males, these factors may offset the negative consequences of nest re-entry denial for their mates.

The ability of dominant females to ensure that their daughters are produced before those of their nestmates means that fitness through daughters is not just a function of the number of daughters produced. That is, fitness may not be accurately reflected by reproductive skew alone. However, the question then arises as to why subordinate females remain in the nest as uninseminated females, rather than leave the colony to found their own nests. We will now argue that this situation of bequeathment by dominants and acquiescence by subordinates is linked to the mandatory nature of group living in *E. robusta*.

In *E. robusta*, per capita brood production increases markedly with the number of adult nestmates (Schwarz 1988) and is largely attributable to the prevention of total brood loss, which is common in single-female nests (Schwarz 1994). Most newly founded and re-used nests contain more than one female, and less than 4% of females nest solitarily during brood rearing, suggesting that solitary nesting entails very low fitness and that

group living is more-or-less mandatory (Schwarz 1994). If guards can control membership of a group then, for subordinates, the costs of becoming inseminated and being subsequently denied colony membership may be very large. High levels of reproductive skew in *E. robusta* are therefore consistent with optimal skew models (Keller & Reeve 1994; Reeve & Keller 1995), which incorporate strong constraints to independent reproduction and where group membership is controlled by a dominant. Our findings are interesting because they suggest that the ability to assume dominance can be passed on behaviourally to daughters, so that effective skew is greater than suggested by offspring numbers or size alone. At the same time, subordinates will realize indirect fitness benefits by increasing colony output (Schwarz 1988), and the possibility of becoming secondary reproductives or dispersing foundresses may provide staying incentives (Reeve & Ratnieks 1993) for them to remain uninseminated within their nests during autumn and winter.

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