Insectes Sociaux

Research article

Strong constraints to independent nesting in a facultatively social bee: quantifying the effects of enemies-at-the-nest

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Abstract. Constraints to independent nesting play a key role in the understanding of social evolution in insects, but the source and the magnitude of such constraints are poorly known for many key taxa. In allodapine bees it is known that solitary nesting females have low rates of successful brood rearing and that this drives selection for cooperative nesting. It has been hypothesized that these constraints are due to the presence of enemies-at-the-nest, such as ants, but no direct link has been demonstrated between such enemies and colony failure. We set up an experiment in which solitary founded nests of an Australian allodapine bee, *Exoneura nigrescens*, were either protected from non-flying predators or left unprotected, and compared the resulting colony survival and brood production rates. We found that protected colonies have much higher rates of survival and that the constraints to independent nesting are extreme, with a mean of less than one offspring per nest at the end of the brood rearing period. This means that cooperative nesting is essential for this species to persist in its habitat.

Keywords: Ecological constraints, social evolution, Allodapini, reproductive skew, ants.

Introduction

Living in social groups entails two opposing selective pressures, competition within groups for limited resources available to members of that group, and the benefits that group living may provide. There has been substantial work in trying to understand how these opposing tensions may have lead to the various social systems that we see in nature. Reproductive skew theories (Reeve and Keller, 2001) currently form our most powerful means for dissecting evolutionary pressures that may have shaped social organizations, but attempts to assess them are beset with significant problems. One major problem is measuring key parameters. Although one important parameter, relatedness among interactants, is more-or-less tractable because of the utility of microsatellite loci, ecological parameters are equally important, but measuring them is often difficult.

One of the major ecological parameters in skew theories involves constraints to independent nesting (Reeve and Keller, 2001). Constraints to independent nesting have long been appreciated (e.g. Lin and Michener, 1972; Queller, 1994) and if they are severe, individuals may be forced to join groups, even if this entails low relatedness and risks of high reproductive skew. Yet for social insects such constraints can be very difficult to measure, and this is especially true when such constraints are thought to derive from predators or parasites. Firstly, these constraints can only be assessed for species where individuals are able to adopt solitary or cooperative strategies. Secondly, one must be able to recognize adoption of those strategies at the outset of brood rearing and then measure their consequences at the end of rearing. Lastly, one must be able to ascribe any differences in outcomes (e.g. colony survival) to predators or parasites, rather than other factors such as extranidal mortality and group-size related variation in foraging efficiency.

Allodapine bees are useful for studies in social evolution because of their wide range in forms of sociality, both within and between species, and also because they are amenable to large scale sampling and field experimentation (Schwarz et al., 1998). Importantly, sociality has not been lost in any extant lineages despite an origin

of sociality more than 40 mya and ubiquitous female totipotency in all clades (Chenoweth et al., in press). Chenoweth et al. (in press) argued that this absence of reversions to strictly solitary living was due to vulnerability of brood to predators in the absence of alloparents.

In allodapine bees, brood are not enclosed within cells, but reared in simple open tunnels in dead stems. The lack of cells makes immatures highly vulnerable to enemies-at-the-nest, especially ants (e.g. Cane and Michener, 1983). Adult females have adaptations to defend their brood, and these seem to be very effective against ants (Cane and Michener, 1983), but solitary females are unable to defend their nest when foraging. This creates potential benefits for cooperative nesting if adult nestmates coordinate their tasks, since one female could guard brood while others forage. Studies on Australian, African and Malagasy allodapines have shown large benefits to cooperative nesting (reviews in Schwarz et al., 1998, 2007). A large component of these benefits involves increased survival of colonies (Hogendoorn and Zammit, 2001), and avoidance of total brood loss (Schwarz et al., 2007). However, the role of enemies-at-the-nest as the cause of colony and brood failure has been purely inferential so that possible confounding factors, such as group-size related food acquisition, could not be ruled out.

Bull and Schwarz (1996) have shown that one potential constraint to independent nesting in the allodapine bee *Exoneura nigrescens*, lack of suitable nesting substrate, is unable to explain cooperative nesting in this species. Here, we use a field experiment to quantify the effect of non-flying predators on colony survival and brood production in solitary nesting females in the same species, and compare this to colonies where the only difference is artificial emplacement of a protective barrier that excludes non-flying predators. Sociality, life history and sex allocation (Silberbauer and Schwarz, 1995; Schwarz, 1994; Bull et al., 1998) have been studied for this species (referred to as a heathland population of *Exoneura bicolor* in earlier publications) in the same habitat used for our experiment, and indicate that females of this species are able to solitarily found new nests, remain with their adult nestmates to cooperatively breed, cofound new nests with relatives, or join the nests of unrelated females. This means that females have a wide range of social and independent options available.

Materials and methods

We conducted a field experiment to determine the effect of ant predation on colonies of *Exoneura nigrescens*. The biology of this species is described by Silberbauer and Schwarz (1995). To generate newly founded colonies, 700 dead flower scapes of the grass-tree *Xanthorrhoea minor*, the main nesting substrate used by this species, were set out in Cobbooboonee State Forest, Victoria, during early autumn (5 March 1999) which represents a key dispersal phase. These were examined in winter (24 June 1999) and 224 bee nests were retrieved. Nests were X-rayed (see Hogendoorn and Zammit, 2001 for methods), revealing a total of 100 nests with a single allodapine occupant, and returned to the field within two days.

Single-female nests were assigned to a treatment ($n = 50$) or control group ($n = 50$), and set out the following morning in Cobbooboonee State Forest. Each treatment nest was protected from ants as follows: Two polyurethane cups were formed into an open 'barrel' with an overhanging apron by cutting one cup shorter and inserting it into the inverted, intact cup. Holes were punched into the centre of each cups' base and slid onto the scape 20 cm below the nest entrance. Tree Tanglefoot Pest Barrier® was applied to the portion of the scape in the barrel and onto the inside of the apron and cup base. This permanently sticky material stopped access by ants along the scape or over the barrel, and the double cup barrel ensured durability of the tanglefoot by shielding it from dust and rain.

The experimental area was cleared of *Xanthorrhoea* scapes, so that no allodapine colonies or nesting material was present in a radius of approximately 200 m. Nests were inserted into *Xanthorrhoea* tussocks in an area of approximately 20 m radius in the centre of the cleared zone, avoiding contact with vegetation above the ant exclusion zone on the treatment nests. Two protected and two control nests were placed in each tussock ($n = 25$) to assess any effect of habitat patchiness in predation risk.

Nests were re-collected in summer (6 Jan 2000) before brood were fully mature. Nests were collected in early morning before extra-nidal activity began, placed on ice and returned to the laboratory for opening, when all contents were preserved. We recorded the number of adults, callows, eggs, small (1st and 2nd instars), medium (3rd and early 4th instars), and large larvae (late 4th instars and prepupae), and the number of pupae.

Results

Of the 100 original nests, 46 control and 44 ant-protected nests were recovered. Seven of the protected nests were compromised in their ability to exclude ants, either because the nest was dislodged, or because tussock growth created a bridge above the exclusion barrier. Compromised nests were removed from analyses, as were nests containing bees other than *E. nigrescens* ($n = 3$).

Micro-habitat (tussock) effects were assessed using a Kruskal-Wallis test on protected and control nests pooled across each tussock. No tussock effect was found on nest occupancy rates ($P = 0.26$), the number of females in nests ($P = 0.40$), or on total brood present ($P = 0.43$). This suggested a lack of tussock effects, and all control and protected nests were subsequently pooled across tussocks.

Table 1 summarises the number of abandoned/destroyed, 1-female, and multi-female nests recovered. A Fisher Exact test showed that females in control nests were more likely to have disappeared from their nest (either through death or desertion) than females from protected nests ($P = 0.001$). The presence of more than one adult female in nests must have resulted from females joining existing colonies since newly emerged females at this time were recognisable by callow pigmentation and lack of wing wear. A Fisher Exact test showed that among all non-compromised scapes that were recovered, protected nests were more likely to contain one or more joiners than control nests ($P = 0.009$, Table 1). Because nests placed into tussocks were randomised from collections over a very large area, it is very unlikely that joiners would have been related to the female whose nest they entered.

Table 1. Occupation status of control ($N = 50$) and ant-excluded ($N = 50$) nests recovered in summer that had been solitarily founded by *E. nigrescens* in winter. Percentages of empty, 1-female, and multi-female nests are given in parentheses.

	Total recovered	Empty	1-female	Multi-female
Control nests	44	26 (59%)	17 (38.6%)	1 (2.3%)
Treatment nests	36	8 (22.2%)	20 (55.6%)	8 (22.2%)

Brood ranged in age from eggs to callows and only nests with adult females contained brood. All callows were female, and male pupae were only found if female pupae or callows were also present. This is consistent with findings of protogyny and female biased ratios for exoneurine allodapines (Schwarz et al., 1998, 2007). Figure 1 shows the number of brood present in protected and protected nests, with these categories further broken down into nests with and without joiners. The mean number of brood over all nests recovered (i.e both occupied and empty combined) was 0.64 ($n = 44$) for control nests, and 2.86 ($n = 36$) for protected nests, and these means are significantly different ($t_{78} = 3.38$; $P = 0.002$). Mean brood size was also significantly different if only occupied nests were considered (mean $= 1.56$, $n = 18$, for control nests, and 3.55, $n = 29$, for protected nests, $t_{45} = 2.54$; $P = 0.02$). Although the mean number of brood in 1-female nests did not differ between protected and control nests ($t_{36} = 1.45$; $p = 0.16$), a Moses extreme reaction test (Siegel and Castellan, 1988) showed the upper range in brood number differed significantly for both the observed control ($p = 0.023$) and for a trimmed control ($p < 0.001$), with protected nests having a greater proportion of very large broods.
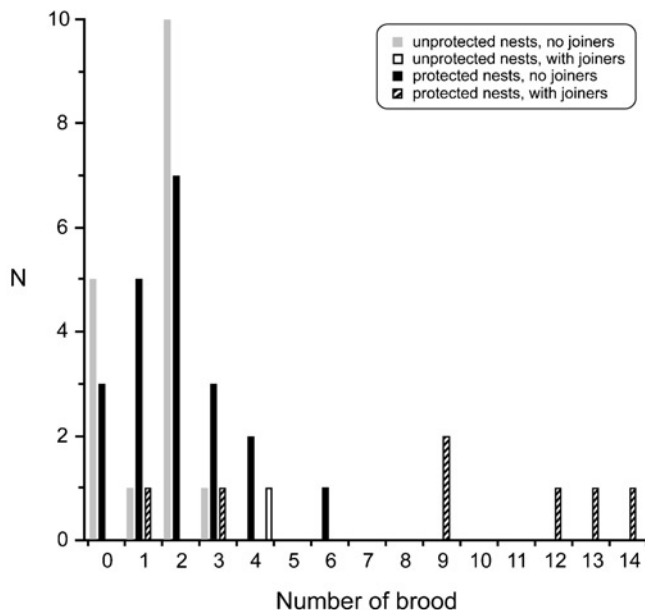
We also examined whether nest joining lead to greater levels of per capita brood production (PCBP) in protected nests (Fig. 2). PCBP, based only on nests with at least some brood, was significantly higher in 2-female than in 1-female protected nests ($t_{26} = 3.05$, $p = 0.005$).
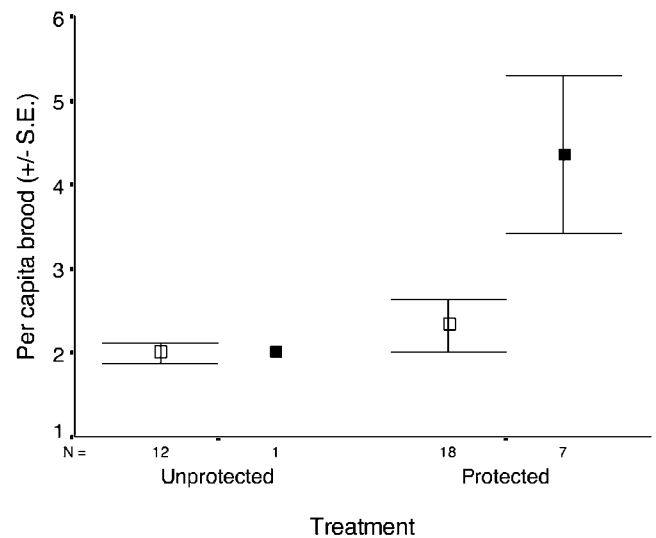

Figure 2. Mean (± 1 standard error) per capita brood production in solitarily founded *E. nigrescens* nests that were either protected or unprotected from ants. Hollow squares are nests with only one adult female at the end of the experiment and black squares represent nests with joiners. Only nests with brood were included when calculating means.

Figure 1. Histograms of final brood numbers in protected and unprotected nests with (open bars) and without (closed bars) joiners.

Discussion

Constraints to independent nesting: Our results unambiguously show that nests of *Exoneura nigrescens* that are protected from non-flying predators have substantially higher rates of survival than unprotected nests. We are not able to determine whether the disappearance of bees from unprotected nests was due to death by predation, or whether bees may have abandoned their nests, possibly following interactions with predators or after predators removed brood. Our results also indicate that some bees joined existing colonies and most nests with joiners had been protected by ant-proof barriers. These joiners could have come from nests in our experiments, since naturally occurring nests had been removed from the study site. While joining rates may reflect a preference to join nests that have not been exposed to predators, it may also be a simple consequence of within-patch dispersal combined

with our experimental design, in that any female abandoning an unprotected nest and attempting to join another nest within its tussock group is twice as likely to encounter a protected nest.

Most previous studies on allodapine species in Australia, Africa and Madagascar (Schwarz et al., 2007; Joyce and Schwarz, 2007) indicate that multi-female colonies have lower rates of total brood failure than single-female colonies, and this provides a substantial benefit for cooperative nesting. Those studies inferred that failure was caused by brood loss to enemies-at-the-nest, but were unable to demonstrate a firm causal link. Our results provide the first direct evidence to show that non-flying predators are indeed a major cause of colony destruction or nest abandonment. Data from pitfall traps in our study site (Zammit, unpubl.) indicate that ants are the only likely non-flying predators, and previous work on allodapines have also implicated ants as the major enemy-at-the-nest for allodapines (e.g. Cane and Michener, 1983; Schwarz et al., 1998).

The effect of ants on colony failure in our study was very large, as less than half of the control nests survived until summer, and the mean number of offspring in these nests was less than one per nest when calculated over all single female control nests at the start of the experiment. This poses a similar constraint to stem-nesting halictine bees studied in Central America (Smith et al., 2003, 2007) but is higher than for ground nesting halictines in the same region as our study (Kukuk et al., 1998). Interestingly, ants were found to be the major predators in those studies as well.

The very low rate of successful brood rearing in our unprotected nests is indicative of a very considerable constraint to independent nesting. Including only the nests that survived, there was no difference in the reproductive output of protected and unprotected single female nests. This indicates that the constraints to solitary nesting posed by enemies at the nest is the predominant factor favouring cooperative breeding. Our experiment only considered the fate of single-female nests successfully occupied over winter, and it is likely that single female colonies established prior to winter would have already experienced predator pressure before this time, adding further constraints to solitary foundation. Our findings are therefore concordant with previous inferential studies on allodapines but for the first time indicate the source (non-flying enemies-at-the-nest) and the degree of constraint.

Hogendoorn and Zammit (2001) showed that in *Exoneura nigrescens* 2-female colonies were almost twice as likely to survive as solitary females, while for 3-female colonies the success was over twice that of single-female nests. This indicates that cooperative nesting is an effective way to counter high predation risks at the nest. Interestingly, our results suggest that females who may have been exposed to predators in unprotected nests are the likely source of joiners in protected nests, so that joining unrelated females may be a fall-back strategy if exposure to predators is high.

Consequences for cooperation: Because the mean number of brood in solitarily-founded unprotected nests is < 1, it is clear that if breeding strategies did not include cooperative nesting the species would be unable to persist in this habitat. This means that cooperative nesting, at some point in the life cycle, is a required strategy for *Exoneura nigrescens* and that this necessity is imposed by the environment rather than by intra-specific competition.

Our per capita brood production data indicated that PCBP is greater in nests with joiners than in single-female nests. This means that if reproduction is shared equally among owners and joiners, then both would benefit from the association. However, Langer et al. (2004) indicated that a Tug-of-War reproductive skew model (Johnston, 2000) is likely to apply to reproductive skew in *E. nigrescens*, so that joining nests is likely to entail unequal reproductive apportionment. This suggests that joining behaviour will pose risks for both joiners and owners, but the extreme constraints to independent nesting shown here may outweigh these risks. In fact, the actual constraints operating on this species are likely to be even higher than our data indicate because brood from single-female unprotected nests in our experiment would have further mortality risks as adults for the six months (January to June) not covered by our study.

One of the most common constraints to independent nesting, either inferred or assumed, is habitat saturation whereby opportunities for independent reproduction are determined by intra-specific factors (e.g. Arnold and Owens, 1999; Hatchwell and Komdeur, 2000; Kokko and Lundberg, 2001). In such situations, it may not be appropriate to set constraints at a fixed value in skew models (Kokko, 2003), since the constraint will vary with what other individuals are doing or with population dynamics, and this may mean that common formulations for many skew models may be invalid for many species. Our study indicates a hard environmental constraint to independent nesting, set by predation pressure rather than intraspecific interactions, and this is concordant with Bull and Schwarz's (1996) study of *Exoneura nigrescens* showing that cooperative nesting is not driven by habitat saturation and their suggestion that it is instead due to survival or productivity benefits of cooperation.

Whether or not constraints to independent nesting are set by the environment or by intra-specific interactions will impact on the applicability of skew theories to particular organisms (Kokko et al., 2002). For example, the adaptive variation in sex allocation with population density in Seychelles Warblers (Komdeur et al., 1997), where habitat saturation drives helper strategies, is likely to differ very strongly from species like *E. nigrescens* where a major ecological constraint does not depend on conspecifics. Comparative studies exploring these issues

are important if we are to determine the generality of current skew models.

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