

KIN ASSOCIATION DURING NEST FOUNDING IN THE BEE  
*EXONEURA BICOLOR*: ACTIVE DISCRIMINATION,  
PHILOPATRY AND FAMILIAR LANDMARKS.\*

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INTRODUCTION

Hamilton's Kin Selection Hypothesis requires, as a minimal condition, that altruistic individuals tend to direct aid towards other individuals sharing a greater genetic similarity than would be expected by random association. Interaction between kin can arise in two ways: (i) It can arise contextually without requiring any discrimination between individuals. For example, low dispersal rates of siblings and population subdivision can lead to interaction between kin at greater rates than would otherwise be expected. (ii) It can arise through recognition mechanisms such that individuals are able to assess their kinship or prior familiarity with other individuals and adopt behavioural strategies accordingly.

Over the last decade there have been an increasing number of studies investigating the mechanisms for kin recognition in social organisms (Gamboa *et al.* 1986; Fletcher and Michener 1987; Carlin 1989). Whilst kin recognition in the highly eusocial ants and bees has been the subject of intensive investigations (see for eg. Veeresch *et al.* 1990), most studies on primitively social insects have investigated recognition in either of three contexts: (i) Recognition of nests by their occupants (eg. Foster and Gamboa 1989; Pfennig 1990; Wcislo 1990); (ii) discrimination between familiar and unfamiliar individuals by guards at the nest entrance (see Michener and Smith 1987 for citations); and (iii) behavioural interactions in novel environments, such as 'circle tubes' (eg. Kukuk 1990), binary choice apparatus (Kukuk *et al.* 1977) and flight boxes (eg. Bornais *et al.* 1983). However, few studies of primitively social insects have explicitly investigated kin recognition away from natal nests and where interactions are not forced upon

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\**Manuscript received 15 July 1991.*

subject animals by the experimenter. Such contexts could be particularly useful for kin recognition studies, since there are likely to be few or no pre-existing recognition labels associated with the interaction arena, and there should be a lower likelihood of artefacts due to forced interactions.

*Exoneura bicolor* is a univoltine bee exhibiting a solitary/quasisocial/semisocial colony polymorphism (Schwarz 1986, 1987). The majority of newly built nests are cofounded by groups of up to 8 females, and electrophoretic analysis of these colonies (Schwarz 1987) has shown that relatedness between cofoundresses is moderately high ( $0.597 \pm 0.062$ ;  $b \pm SE$ ). In montane habitats, this bee nests in the dead fronds of tree-ferns, and this means that nests tend to occur in well-defined aggregations.

The finding of moderately high values of relatedness within newly cofounded nests indicates effective kin association between dispersing females in this species. However, it is possible that such association arises without active assessment of kinship or prior familiarity; for example, it could arise if foundresses are strongly philopatric or if foundress dispersal is asynchronous among neighbouring colonies, so that association between previous nestmates arises incidentally. A potential role for philopatry as a factor contributing to relatedness between cofoundresses is suggested by a study by Blows and Schwarz (1991), who found significant coancestries for nesting aggregations in 3 out of 8 localities studied. Statistically significant coancestries varied from  $\Theta = 0.03$  to 0.11. Since cofounding of new nests is limited to an approximately 2 week period in spring (Schwarz 1986), asynchronous dispersal between colonies is unlikely to contribute to relatedness among cofoundresses.

In many *Polistes* species, new nests are often founded by two or more related gynes after overwintering. In these wasps, kin association may result in part from philopatry (Klahn 1979; Noonan 1981) as well as nestmate recognition (Post and Jeanne 1982; Bornais et al. 1983; but see Pratte 1982). In this study we explicitly examine both these possible factors during colony foundation in a primitively social allodapine bee, *Exoneura bicolor*. We present data confirming that cofounding by kin in a primitively social allodapine bee, *Exoneura bicolor*, is the result of active discrimination by females and that this discrimination occurs even in novel

environments where contextual cues, such as natal nests and familiar landmarks, are absent.

#### MATERIALS AND METHODS

Intact colonies of *E. bicolor* were collected from Sherbrooke National Park in late April 1988. Whole nests were plugged with cotton wool and taken to La Trobe University where they were stored at 10°C until released. Adult females were removed from nests by splitting the nests open longitudinally; colony members were transferred to petri dishes and given unique abdominal and thoracic marks using Testors Model Master™ enamel paint. A total of 151 females, taken from 26 multifemale nests were used in the experiment. The number of original inhabitants per nest ranged from 2 to 21 (mean = 5.6; median = 4).

Marked females were released at a series of stakes bearing trap nests in the La Trobe University Zoology Wildlife Reserve. This reserve does not contain naturally occurring colonies of allodapine bees. Trap nests comprised 40 cm lengths of dead tree fern fronds (*Cyathea australis*) tied onto upright metal stakes (black painted star pickets), with 30 fronds per picket. Fronds were fixed in sub-horizontal positions on the stakes. A total of 6 stakes were used, placed in a straight line with stakes at 2 m intervals. On 2 May 1988 bees were released *en mass* by placing all bees into a single container and gently tapping them onto the central stake. While we cannot rule out the possibility that females remained in nestmate clumps in this container, this seems unlikely given the number of females involved, the size of the container (small ice-cream container), and the considerable shaking that occurred when carrying the bees from the lab to the field release site. Bees were released at 1030 hr; ambient temperature was 21°C and there was no wind.

Montane populations of *E. bicolor* do not show foundress dispersal during autumn. Apart from colonies whose nests have been damaged, nearly all nest initiation is restricted to a short period of about 2 weeks in late spring (Schwarz 1986). Therefore, this experiment was carried out at a time when some colonies may need to find new nests, but foundress dispersal is otherwise rare.

After release, bees were left for 2 weeks and collected on 16 May 1988 during a rainy period so that all colony members would be present. Newly constructed nests were plugged with cotton

wool and opened in the laboratory. All recovered females were scored for paint marks and then assayed electrophoretically at 2 presumptive loci, both staining as esterases. Electrophoresis and staining techniques are described in Blows and Schwarz (1991). Relatedness between nestmates was calculated using Goodnight and Queller's (1989) technique based on Grafen's (1985) relatedness coefficient, and can be interpreted as a measure of identity by descent. Relatedness coefficients and their estimates were calculated by jackknifing across colonies. All nest lengths were recorded.

### RESULTS

In the two weeks prior to collection of the newly founded nests, females were observed flying around fern fronds, constructing new nests, or visiting newly established nests. Occupants of new nests were frequently observed sitting in the entrances of their nests with their antennae pointing outwards. Visitors to new nests frequently entered several nests in rapid succession, although most visits lasted for only a few seconds. Visits to occupied nests did not seem to be accompanied by aggression on behalf of either the visitors or the occupants.

Of the 151 bees released, 77 were recovered from a total of 54 nests, comprising 20 multifemale nests and 34 single-female nests. A further three nests did not contain any bees. The number of occupied nests per stake and bees per nest are summarized in Table 1. The greatest number of nests were founded at the central stake on which the bees had originally been released, with progressively decreasing numbers in adjacent stakes. No nests were constructed at either of the terminal stakes.

17 nests contained two bees each, and 3 nests had three bees each. Markings on 37 of the recovered bees were lost, but where all bees in a new nest still had markings (five 2-female nests), all bees nesting together had originally been nestmates (although each pair had originated from different source nests). However, a number of bees nesting alone had original nestmates that were also nesting alone or with unmarked bees at the same stake. Of the 6 nests containing more than one female with intact marks, all marked females were from the same original nests, so that we have no evidence of colony mixing after dispersal.

All females from the 20 new nests containing more than one female were electrophoresed and relatedness was estimated as  $0.32 \pm 0.09$  ( $b \pm SE$ ). This estimate is lower than those reported by Schwarz (1987) but falls within the range of estimates in a more comprehensive study of intra-colony relatedness in natural populations (Blows and Schwarz 1991).

Positions of solitary and multifemale nests relative to the release-point stake were compared using Chi-square tests. Whilst the distribution of single and multifemale nests did not differ significantly ( $\chi^2 = 3.463$ ,  $p > 0.05$ ), the distribution of females in these two nest types differed slightly but significantly ( $\chi^2 = 6.946$ ,  $p < 0.05$ ), with proportionately more females at the distal stakes nesting solitarily. Therefore there is some evidence that females dispersing shorter distances have a greater likelihood of cofounding, or that cofounding females disperse over shorter distances.

Mean nest lengths for single and multifemale nests are given in Table 1. These data show that mean nest length increases synergistically with the number of cofoundresses, so that mean nest lengths per resident female are approximately 3 cm, 4 cm and 4.7 cm for single, two and three-female nests respectively.

#### DISCUSSION

Our results indicate that when original nests of *Exoneura bicolor* were destroyed, and when alternative nesting sites are available at a range of distances from the release site, those females recovered tended to disperse short distances. Furthermore, the majority of bees recovered in our experiment had joined relatives to form multi-female colonies.

Table 1. Mean nest length and the distribution of single and multi-female nests founded among 6 experimental stakes containing trap nests. Stakes were placed in a straight line at 2 m intervals. Bees were originally released at stake 4.

Stake	1	2	3	4	5	6	Nest Length	
							Mean	S.E.
1-female nests	0	5	6	14	9	0	2.97	0.33
2-female nests	0	0	4	10	3	0	8.03	0.79
3-female nests	0	0	1	1	1	0	14.17	2.05
Total nests	0	5	11	25	13	0		

Three factors, which are not mutually exclusive, could explain the limited dispersal distances observed in our experiment: (i) females may prefer to nest in central rather than peripheral sites, perhaps to reduce exposure to predation; (ii) females may be minimizing searching effort by utilizing the first suitable nesting sites encountered; (iii) females may be reducing dispersal distances in order to increase the likelihood of locating previous nestmates.

Given the large number of nesting sites per stake in our experiment (6 stakes with 60 frond-ends per stake) it is unlikely that either low dispersal rates or confounding in our experiment are due to limited nesting sites. In natural populations nest densities may become very high, with up to four nests per frond; nesting densities in our experiment were comparatively low. Therefore, kin association occurred in a novel environment and, although dispersal distances were low, they are not sufficient to explain kin association during nest initiation.

However, cooperatively nesting females tended to be closer to the release point, suggesting that females may have a greater likelihood of nesting with kin if they disperse shorter distances. A similar situation appears to occur in some *Polistes* wasps, where philopatry may bring related gynes together after overwintering, but is not sufficient to explain the extent of observed kin association (Bornais et al. 1983). The lower dispersal distances of cooperatively nesting females could also be explained if there is competition for central nesting sites (to minimize predation) and if cooperatively nesting females are better able to compete for these sites. We feel that this possibility is unlikely for several reasons. Firstly, nesting densities in our experiment were low compared to many natural aggregations, so that possible competition in our experiment is expected to be relatively minor. Secondly, we did not observe any agonistic interactions, either between resident females and visitors, or between residents within stakes. Thirdly, if central areas are preferred because of reduced predator pressure, excluding other females from these areas would tend to increase the exposure of central nesting females to any predators that do enter these areas.

Therefore, our findings suggest that females of *Exoneura bicolor* assort themselves into kin groups (or groups of previous nestmates) on the basis of cues that can be carried into a novel

environment, even when nesting sites are not limiting and a moderately large number of kin groups are present. Whilst we have not strictly shown that such cues are utilized during natural periods of founding, it seems unlikely that the ability to use these cues would not be utilized at times when kin association among large groups of dispersers is frequent. In colonies of *E. bicolor*, reproductivity per female increases with the number of nestmates (Schwarz 1988a) indicating benefits for cooperative nesting, but communal progressive rearing in allodapine bees creates a high potential for nestmate parasitism. These two factors may create strong selective advantages for cooperative nest founding moderated by kin or nestmate recognition (Schwarz 1988b).

Our finding of synergistic increases in nest length with the number of nestmates could indicate some immediate advantages for cooperative nesting, even in the absence of brood rearing. Nests in *E. bicolor* are often quite long (>0.5m) (Schwarz 1986) and it is possible that longer nests may enhance defence against parasites or predators such as ants. However, there may be an alternative explanation for the short nest lengths of single females. Schwarz and O'Keefe (1991) have shown that when kin are not available, most solitary nesting females in autumn eventually join non-relatives to form multi-female colonies. It is possible that solitary females delay investment in nest construction until they are either joined by other females or defect to other colonies.

In conclusion, our results suggest that the high relatedness between females in newly cofounded nests in natural populations are the result of active discrimination by females. Such discrimination could be based on relatedness or prior familiarity but cannot be explained by philopatry or asynchronous dispersal. The experimental methods described here allow kin recognition to be investigated without inducing forced interactions. Our techniques could also be elaborated to investigate recognition in more detail, whilst still avoiding unrealistic scenarios. For example, our protocol could be expanded to test for within-colony discrimination between matriline by using larger source colonies (which would contain several matrilines) and comparing intra-colony relatedness before and after cofounding experiments.

## SUMMARY

Previous work has demonstrated moderately high intra-colony relatedness among cofoundresses in newly built nests of the allopapine bee *Exoneura bicolor*. In this paper we test whether this kin association can be explained by spatially limited or asynchronous dispersal, or whether it requires active kin or nestmate recognition. Bees from 21 colonies were released in a novel environment containing abundant potential nesting sites at various distances from the release point. Most bees recovered had formed multi-female nests. Relatedness among cofoundresses was significant, and we found no evidence of colony mixing after release. Dispersal distances tended to be small (<4 m), and cooperatively nesting females tended to be closer to the release site than solitary females. Kin association during cofounding in this bee requires active recognition of either kinship or previous familiarity, but this could be facilitated by limited dispersal distances.

## ACKNOWLEDGEMENTS

We would like to thank K. J. O'Keefe for help with fieldwork, G. Paras and the La Trobe University Wildlife Reserve for the use of their facilities, and D. C. Queller and K. F. Goodnight for providing us with their programme for estimating relatedness using electrophoretic data. We also thank an anonymous reviewer for helpful comments on an earlier draft of this manuscript. Bees were collected under permit no. 878/54 (Dept. of Conservation, Forests and Land). This work was supported by the National Research Fellowship Scheme and the Department of Zoology, La Trobe University.

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