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Can genetically unrelated individuals join colonies of *Ropalidia marginata* ?

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ABSTRACT

Field and laboratory investigations of the primitively eusocial wasp *Ropalidia marginata* suggest that worker behaviour cannot be explained satisfactorily either by intra-colony genetic relatedness or by parental manipulation. Besides, polyandry and serial polygyny lead to high levels of intra-colony genetic variability. For these reasons, an attempt was made to explore the role of mutualistic interactions in social evolution. Artificially mixed colonies were created introducing 4–12 one day old unrelated female wasps into six semi-natural colonies of *Ropalidia marginata*. Wasps naturally eclosing on the colonies during the period of introduction were used as controls. All introduced animals were accepted without any significant aggression. The introduced animals stayed on their foster colonies as long as the controls did. There was no evidence of any differential treatment or recognition of the introduced animals by the older animals in the foster colonies. Five out of 56 introduced animals went on to become foragers, and brought food to their foster colonies within 25 days of introduction. This is not significantly different from the fact that six out of 38 control animals also became foragers during the same period. More importantly, in two of the six colonies where there were natural queen turn overs, an introduced animal became the replacement queen in both cases. These results lend support to mutualistic theories of social evolution.

INTRODUCTION

Ropalidia marginata (Lep.) (Hymenoptera: Vespidae) is a primitively eusocial polistine wasp abundantly distributed in peninsular India. Although single foundress nests are seen, most nests have several female wasps on them. This is because many new nests are founded by groups of females and also due to eclosing daughters who often stay back on their

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natal nests rather than leave to doubt new ones. In all multifemale nests, at any given time, only one individual is the queen or egg-layer while the remaining are workers who perform the tasks of nest building, maintenance and brood care. It is true, however, that the queens are frequently driven away to be replaced by one of the workers who in turn may be driven away to be replaced by yet another worker and so on [1–3].

The evolution by natural selection of such worker behaviour continues to be a mystery. The haplodiploidy hypothesis argues that worker behaviour might yield more inclusive fitness than a solitary nesting strategy because of the high intra-colony genetic relatedness made possible by the haplodiploid mode of sex determination in the Hymenoptera [4, 5]. Previous studies on *R. marginata* have shown that polyandry (multiple mating by the queen) and serial polygyny (frequent replacement of queens) considerably reduce intra-colony genetic relatedness, so that this seems unlikely [6–9]. Another class of theories suggests that only sub-fertile individuals may become workers, so that their cost of not reproducing is more easily offset by the inclusive fitness obtained as workers [10, 11]. Previous work has shown that although some evidence of subfertility is evident in laboratory conditions, this is not sufficient to explain why so many individuals became workers [12, 13]. Lin and Michener have argued that social insect workers may not be entirely altruistic but may, at least in part, be mutualistic and that an individual may join a multifemale nest because of the hope that it may become a queen some day [14]. Mutualism of course does not require high intra-colony relatedness. Indeed, we already have evidence that due to polyandry and serial polygyny, rather distantly related individuals, — even as distantly related as the worker's mother's cousin's grandchild — eclose on natural colonies. The question of interest then is whether both close and distant relatives are treated alike or distinguished. To make a direct test of this, we created genetically mixed colonies by introducing one day old unrelated individuals onto colonies of *R. marginata*. This introduction simulates natural eclosion of distantly related individuals in natural colonies.

MATERIAL AND METHODS

Six colonies of *R. marginata* with about 10–15 pupae and 10–12 adults each were selected to serve as recipient colonies. These were either naturally initiated or transplanted and maintained for several weeks in a vespiary at the Centre for Ecological Sciences, Indian Institute of Science, Bangalore. All adults were marked with unique colour codes using quick drying paints. Quantitative observations on such nests were carried out for a period of twenty hours prior to introduction of unrelated wasps [15]. Nests with about twenty pupae were collected for the purpose of obtaining unrelated animals for introduction. Care was taken to ensure that the colonies collected for obtaining animals for introduction, were atleast 10 km away from the site of collection or initiation of the recipient nests. The adults, larvae and the eggs were removed, and the nests with only the pupae were maintained at room temperature and monitored daily for eclosion of adults.

Adults eclosing from these donor nests were removed immediately upon eclosion, isolated into 15 ml glass vials and used for introducing onto recipient nests. These adult animals were also marked with unique colour codes, and introduced onto the nests within 24 hours after eclosion. The introduction of unrelated animals was matched with eclosion of adults on the recipient nests so that the matched natal animals could be treated as controls. Introductions were continued till there were atleast six unrelated animals on the foster nest. The period of introduction ranged from 7 to 10 days. Twenty hours of observations were repeated, as before, a week after the introduction of last unrelated animal. The post-introduction observations were carried out in the blind, i.e. the observer was not aware of the identity of the colour codes of the control and experimental animals on the nest. (The number of introduced (experimental) animals, control animals and other details of each of the six nests used in the experiment are given in Table 1.)

Records of the brood composition of the nest and census records of the animals present on them are being maintained at intervals of 1–2 days until the last introduced animal disappears.

Table 1 Genetically mixed colonies of *Ropalidia marginata*

Expt. No.	Recipient Nest Code	No.of older nest animals	No. of young nest animals (Control)	No.of introduced animals (Experimental)
1	L65	3	9	8
2	L39	7	3	6
3	L29	12	12	11
4 *	L76	6	4	11
5	L63	9	7	9
6 *	L52	13	4	11

* In these two nests there was a natural queen turn over and one of the introduced animals became the new queen in both cases.

RESULTS

It was observed that the introduced unrelated animals were accepted without any aggression on all the six experimental nests. Preliminary statistical analysis has shown that there is no significant difference in the time spent in the various behaviours by the natal and the introduced animals. There was no evidence of any differential treatment, or recognition of the introduced animals and the matched control animals, by the older nest animals (Fig.1). The introduced animals were found to stay on the foster colony for as long as the controls did. The control (natal) animals stayed on the nest for an average of 48.3 ± 29.9 days (n = 38) while the introduced unrelated animals stayed on the foster nests for an average of 47.9 ± 29.9 days (n = 56).

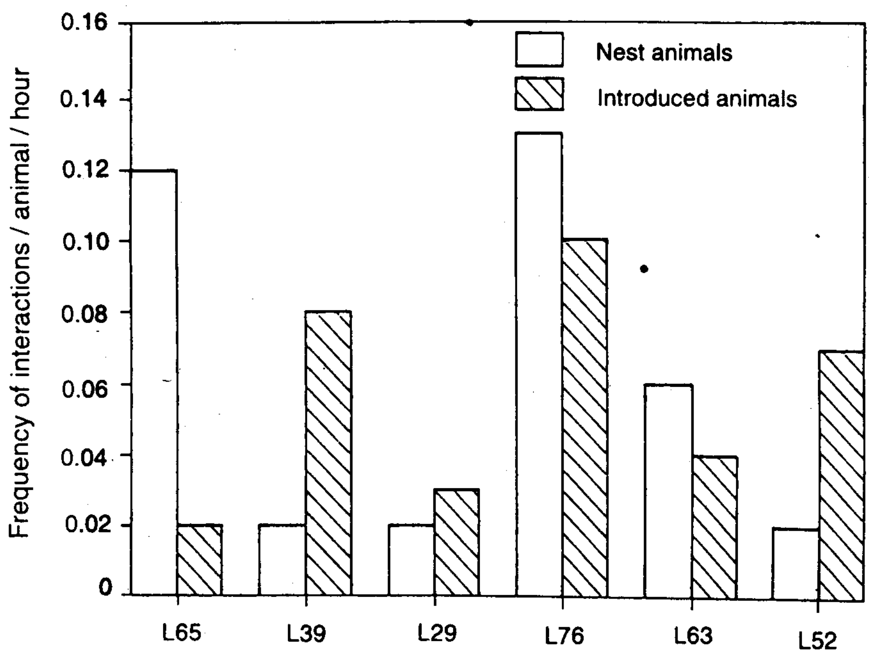

Fig.1 Mean interactions by older nest animals towards younger nest animals (Controls) and introduced (Experimental) are not significantly different from each other (Wilcoxon Matched-pairs Signed-ranks Test $T_s = 10$, $n = 6$, $p > 0.05$).

Foraging and bringing food to the nest is perhaps the most important task of the workers. It was found that five out of 56 introduced animals went on to become foragers, and brought food to their foster colonies within 25 days after introduction while six out of 38 control natal animals foraged and brought food in the same period. These numbers are not significantly different from each other ($G = 0.964$, $P > 0.05$), indicating that the introduced animals were integrated as a part of their foster colonies and performed tasks similar to their matched natal counterparts.

Another interesting and important observation was that in two out of the six colonies observed so far, there were natural queen turn overs and in both the cases an introduced animal became the replacement queen. This happened in spite of the presence of 5 old nest animals, 4 young nest animals and 10 other introduced animals in the nest L76, and 12 old nest animals, 4 young nest animals and 10 other introduced animals, in the nest L52. As on 9-4-93 the two introduced animals which have become queens have remained so for 110 days and 51 days respectively in L76 and L52 and continue to do so. This reiterates the observation that the unrelated animals are being accepted on the foreign nests without any apparent differential treatment.

DISCUSSION

R. marginata females are known to be capable of discriminating nestmates from non-nestmates outside the context of their nests. To do so however, both the discriminating as well as the discriminated animals need to be exposed to a fragment of their nest and a subset of their nestmates. We had interpreted this to mean that recognition labels and templates used in nestmate discrimination are acquired/learned after eclosion, from a common source — the nest or the bodies of the nestmates — so that it is unlikely that different levels of genetic relatedness within a colony can be discriminated [16, 17]. The present results showing that one day old unrelated non-nestmates are readily accepted by colonies and further that these introduced animals become well integrated into their foster colonies with no evidence of differential treatment towards them as compared to natal animals, supports our earlier interpretation.

Even more significant is our finding that the introduced animals become foragers as frequently as nest animals, and that they become replacement queens in their foster colonies. The last mentioned result has other important implications as well. Although foreign animals are seldom known to join mature colonies (this has been seen occasionally however) we have seen atleast 69 cases of apparent foreigners joining pre-emergence colonies (Shakarad and Gadagkar, unpublished observations). Besides, rather distantly related individuals routinely eclose on natural colonies due to polyandry and polygyny. We believe that our experiments simulate these natural phenomena. The fact that such introduced animals can go on to become queens in their foster colonies lends credence to mutualistic theories and the idea that workers may work because of the hope of becoming future queens, rather than because of their inability to reproduce (parental manipulation/subfertility hypothesis) or because of the opportunity to rear genetically closely related brood (haplodiploidy hypothesis).

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