The Effect of Queen Number on Nestmate Discrimination in the Facultatively Polygynous Ant Pseudomyrmex pallidus (Hymenoptera: Formicidae)

Philip T. Starks, Rebecca E. Watson, Matthew J. DiPaola & Christian P. DiPaola


Abstract

We present evidence indicating that the level of nestmate discrimination in the facultatively polygynous ant Pseudomyrmex pallidus varies predictably with the number of queens in the colony. P. pallidus workers were introduced into observation arenas in either nestmate or non-nestmate pairs. During the 5-min period immediately following the first contact between test ants, all interactions and relative distances were recorded. Aggression between non-nestmates was negatively correlated with the number of queens in the colony and distance between nestmates was positively correlated. These results are consistent with predictions of Reeve's (1989) optimal acceptance threshold model.

Corresponding author: Philip T. Starks, Section of Neurobiology and Behavior, W311 Seeley G. Mudd Hall, Cornell University, Ithaca, NY 14853—2702, USA. E-mail: pts3@cornell.edu

Introduction

Hamilton (1964a,b) demonstrated that altruism will be selectively favored when the fitness cost to an altruist is lower than the product of the recipient's fitness benefit and the level of relatedness between the interactants. An organism can therefore maximize the genetic payoff of altruism by directing altruism at highly related kin (Holmes & Sherman 1983). As predicted, the differential treatment of kin and non-kin has been documented in many social animals (reviewed by Fletcher & Michener 1987; Hepper 1991; Sherman et al. 1997).

Kin (or nestmate) discrimination has been extensively studied in the social Hymenoptera (reviewed by Fletcher & Michener 1987). Although the ability to discriminate nestmates from non-nestmates is common, the level of discrimination varies in strength between species (Wilson 1971) and may be strongly dependent on context (Starks et al. 1998). In ants, for example, nestmate discrimination can vary with colony-specific factors, such as colony size (Stuart 1991), stage (Balas & Adams 1996) and queen number (Janzen 1973; Keller & Passera 1989) in addition
to environmental factors, such as time of the year (Ichinose 1991) and proximity to other colonies (Heinze et al. 1996).

Reeve (1989) proposed the optimal acceptance threshold model to help explain context dependence. The model assumes that an individual develops a recognition template, compares the recognition cues of encountered conspecifics to this template and determines whether the conspecific is kin based on the degree of template-cue dissimilarity. Reeve further suggested that, based on the degree of template-cue dissimilarity, a threshold exists above which conspecifics are rejected (aggressed against) and below which they are accepted (treated tolerantly; Fig. 1). Acceptance thresholds are predicted to balance optimally the fitness costs of wrongly accepting an undesirable conspecific (non-kin) or rejecting a desirable conspecific (kin). Thus, acceptance thresholds should shift in contexts that influence the relative fitness costs of accepting non-nestmates (acceptance errors) and rejecting nestmates (rejection errors).

We examined nestmate discrimination in the facultatively polygynous ant

![Diagram](image)

**Fig. 1:** Frequency distributions of dissimilarity between an actor's recognition template and an invader's recognition cues. T1 and T2 are optimal thresholds above which individuals are rejected (aggressed against) and below which individuals are accepted (treated tolerantly). A. Acceptance threshold for a monogynous colony; B. acceptance threshold for a polygynous colony. Within-colony genetic diversity broadens the range of nestmate template-cue dissimilarity in polygynous colonies relative to monogynous colonies. Thus, for any threshold, the probability of a rejection error in polygynous colonies is greater than or equal to that of monogynous colonies. To balance the cost of acceptance and rejection errors, the acceptance threshold for polygynous colonies is predicted to shift and become less restrictive, thus increasing the probability of acceptance errors relative to monogynous colonies.

Adapted from Reeve (1989)

*Pseudomyrmex pallidus*. Previous evidence suggests that several *Pseudomyrmex* spp. can discriminate between nestmates and non-nestmates (Janzen 1973; Mintzer 1982; Mintzer & Vinson 1985; Jaffé et al. 1986). However, Janzen (1973) reported that the obligate polygynous ant *Pseudomyrmex venefica* did not display behavioral discrimination, whereas the obligate monogynous ant *Pseudomyrmex ferruginea* did discriminate between nestmates and non-nestmates. Examining species which can adopt either founding strategy may reveal information about factors underlying this difference in levels of nestmate discrimination.

Because the suite (or gestalt) of recognition cues typically incorporates both environmental and genetic components (reviewed by Fletcher & Michener 1987), our study animals were maintained in uniform environments, thus minimizing the environmental cue diversity. Endogenous cues in *Pseudomyrmex* have been shown to be produced by workers (Mintzer 1982; Mintzer & Vinson 1985; Jaffé et al. 1986). Therefore, because of multiple matrilineages, and thus an increased intracolonial genetic diversity, we predicted that the distribution of dissimilarity between recognition templates and cues would be broader for nestmates in polygynous colonies than for nestmates in monogynous colonies (Fig. 1). Accordingly, the degree of overlap between the distributions of nestmate and non-nestmate recognition template-cue dissimilarity should be greater for polygynous colonies than for monogynous colonies (Fig. 1).

Using the framework of the optimal acceptance threshold model, we predicted that workers from both polygynous and monogynous colonies would discriminate between nestmates and non-nestmates. However, relative to workers from monogynous colonies, workers from polygynous colonies should (1) make more acceptance errors due to a right-shifted acceptance threshold and (2) make a greater than or equal number of rejection errors because of the broader distribution of cue-template dissimilarity (Fig. 1). Workers from polygynous colonies should therefore appear to have a less well-developed ability to discriminate nestmates from non-nestmates.

Workers from field-captured monogynous and polygynous *P. pallidus* colonies were introduced into an observation arena in dyads of either two nestmates or two non-nestmates. We recorded all aggressive interactions and data on spatial associations, since nestmate discrimination has been detected with both types of data (reviewed in Fletcher & Michener 1987; Hepper 1991).

**Methods**

**Source of Ants**

Twenty-four *P. pallidus* colonies, each separated by more than 5 m, were collected from Jack Brooks Park, TX, USA, by J. Peters, between 07:30 and 14:00 h on May 23, 1996 (n = 9 colonies) and Dec. 6, 1996 (n = 15 colonies). Colonies were located by breaking open stems of various weeds and large grasses; if ants or brood were visible, the stem was cut at ground level, placed in a ziplock bag, and stored in a cooler at 10–2°C. Although they often contained ants, adjacent stems
were not collected because it was unclear whether these represented separate colonies. On the day of collection, colonies were transported to Rice University and anesthetized using either brief exposure to ice or CO₂. The stem was split lengthways to remove all ants and brood, which were later shipped to Cornell University, Ithaca, NY, USA, in 50 ml Falcon tubes.

Each colony was housed in a nest made of two glass slides (7.5 × 2.5 cm each) separated by a 0.2 mm thick balsa wood spacer. The nests were maintained in plastic containers (13 × 13 × 5 cm) containing water, honey-water, and pieces of cricket (replenished every 2–3 days). All colonies were maintained at ≈ 30°C day/20°C night under a normal light:dark cycle. Colonies were censused twice per week. Colonies containing two or more physogastric individuals with wing scars were considered polygynous and colonies with a single physogastric individual with wing scars were considered monogynous. Recent evidence from analysis of microsatellite genotypes demonstrates that the number of physogastric queens present is the best predictor of the number of matrilines in a colony (Peters 1997; Peters, unpubl. data).

Interaction Observations

Observations of dyads consisting of either two similarly sized nestmate workers or two similarly sized non-nestmate workers took place between 0900 and 1200 h each Saturday from Feb. 1, 1997 through Mar. 1, 1997 in Seeley Mudd Hall, Cornell University.

In experiment 1, non-nestmate dyads consisted of a mix of individuals from monogynous and polygynous colonies. Only one nestmate pair was sampled from each colony and no two colonies were used in pair-wise combinations more than once.

In experiment 2, non-nestmate dyads were matched for the number of queens in the colony to allow us to test for effects of queen number on nestmate discrimination. For each queen number, the number of colonies and average (±SE) number of workers were: one queen (n = 6, 38.33 ± 6.67), two queens (n = 4, 50.0 ± 10.20), three queens (n = 5, 47.60 ± 7.16), four queens (n = 4, 44.25 ± 6.99) and eight queens (n = 5, 55.00 ± 5.48). For nestmate dyads, colonies were sampled from multiple times (range 2–5, 2.70 ± 0.21). For non-nestmate dyads, no colonies were used in pair-wise comparisons more than twice (range 1–2, 1.40 ± 0.07). Between colonies of equal queen number, there was no observable difference detected in nestmate or non-nestmate interactions, thus all dyads were treated as independent regardless of source colony identity.

In both experiments, trial ants were placed in a Petri dish (Falcon, 100 × 20 mm), which rested on white paper with a numbered grid (cm²). Observations began immediately after the first interaction and lasted 5 min. Location (i.e. grid number) of each individual was recorded every 30 s (10 observations per trial). All aggressive activities were recorded using a modified Carlin & Hölldobler (1986) four-point aggression scale (Table 1). Because test ants were not returned to their colony, each ant was observed only once. All arena observations were taken by two individuals unaware of the test animals’ nest affiliations. Petri dishes were washed with soap and warm water between trials. A total of 163 trials were carried out.

Table 1: Behavioral units and aggression scores (0–3) used to assess nestmate discrimination (modified from Carlin & Hölldobler 1986)

<table>
<thead>
<tr>
<th>Score</th>
<th>Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Mutual tolerance, huddling, grooming, antennation</td>
</tr>
<tr>
<td>1</td>
<td>Avoidance, jerking back, aggressive antennation</td>
</tr>
<tr>
<td>2</td>
<td>Nipping, pulling, carrying, open mandible display</td>
</tr>
<tr>
<td>3</td>
<td>Locked together, biting, stinging</td>
</tr>
</tbody>
</table>

Statistical Analyses

We calculated the mean distance between individuals as the average of the 10 straight-line distances between the centers of each grid square occupied by the test ants. A single aggression score was calculated for each trial: the median value of all aggressive interactions between individuals in a dyad. Acceptance errors are defined as extreme tolerance between non-nestmates (i.e. an aggression score of 0) and rejection errors are defined as extreme aggression between nestmates (i.e. an aggression score of 2–3). Comparisons were made as a function of colony type (monogynous or polygynous) and number of queens in the colony. All descriptive statistics are means ± standard errors. Parametric tests were performed on distance and interaction data since the data appeared normally distributed. Aggressive interaction data were skewed and thus nonparametric tests were used.

Results

Experiment 1

Nestmate discrimination

Non-nestmates in dyads were significantly more aggressive than nestmates in dyads (non-nestmates, 1.69 ± 0.31, n = 16; nestmates, 0.37 ± 0.19, n = 15; Mann–Whitney test, W = 168.5, p < 0.01), thus confirming nestmate discrimination abilities.

Experiment 2

Number of interactions

Nestmate vs. non-nestmate dyads: Significantly more interactions occurred between non-nestmate workers than between nestmate workers from both mono-
gynous (unpaired t-test, $t_{45} = 3.30, p < 0.01$; Fig. 2) and polygynous colonies ($t_{5} = 5.18, p < 0.001$; Fig. 2).

**Polygynous vs. monogynous colonies.** Significantly more interactions occurred between non-nestmates from polygynous colonies than between non-nestmates from monogynous colonies ($t_{10} = 2.20, p < 0.05$; Fig. 2). Interactions were not more likely to occur between nestmates from polygynous colonies than between nestmates from monogynous colonies ($t_{10} = 0.83, p = ns$; Fig. 2).

**Aggressive interactions**

**Nestmate vs. non-nestmate dyads.** Aggression was significantly greater between non-nestmate workers than between nestmate workers from both monogynous ($W = 407.0, p < 0.001$; Fig. 3) and polygynous colonies ($W = 1280.0, p < 0.01$; Fig. 3).

**Polygynous vs. monogynous colonies.** Workers from monogynous colonies were significantly more aggressive to non-nestmates than were workers from polygynous colonies ($W = 1047.0, p < 0.05$; Fig. 3). The level of aggression did not differ between nestmates from polygynous colonies and between nestmates from monogynous colonies ($W = 847.0, p = ns$; Fig. 3). Aggression between non-nestmates correlated weakly with the number of queens in the colony (linear regression, $R^2 = 8.5\%, F = 5.96, df = 65, p < 0.05$; Fig. 4).

![Graph showing mean number of interactions between worker nestmates and non-nestmates from monogynous and polygynous colonies.](image)

*Fig. 2.* Mean number of interactions between worker nestmates (open columns) and worker non-nestmates (filled columns) from either monogynous or polygynous colonies. Columns are means (± SE). Sample sizes are listed under the appropriate column. ns, $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

![Graph showing mean aggression scores between worker non-nestmates and the number of queens in the colony.](image)

*Fig. 4.* Regression of mean aggression scores between worker non-nestmates and the number of queens in the colony. Squares are means (± SE). Sample sizes: monogynous $n = 26$, polygynous $n = 10$ each.
Distance between individuals

_Nestmate vs. non-nestmate dyads_. Average distance between nestmates from both monogynous (t_{26} = 2.46, p < 0.05; Fig. 5) and polygynous (t_{56} = 7.88, p < 0.001; Fig. 5) colonies was greater than the distance between non-nestmates. This result stems from the close proximity necessary for the aggressive interactions more common in non-nestmate dyads.

_Polygynous vs. monogynous colonies_. Average distance between non-nestmates from monogynous colonies did not differ from the distance between non-nestmates from polygynous colonies (t_{46} = 1.35, p = ns; Fig. 5). However, nestmates from monogynous colonies associated more closely than did nestmates from polygynous colonies (t_{46} = 2.86, p < 0.01; Fig. 5). The number of queens in a colony positively correlated with the distance between nestmates (R^2 = 22.7%, F = 18.77, df = 65, p < 0.001; Fig. 6).

Acceptance and rejection errors

Acceptance errors were significantly more likely between individuals from polygynous colonies than between individuals from monogynous colonies (χ^2 = 6.77, p < 0.01; Fig. 7). No significant difference between colony types in rejection errors was observed (χ^2 = 1.31, p = ns; Fig. 7).

**Fig. 6**: Regression of the mean distance between worker nestmates in dyads and the number of queens in the colony. Squares are means (+SE). Sample sizes are: monogynous n = 26, polygynous n = 10 each.

**Fig. 5**: Mean distance between individuals in dyads consisting of worker nestmates (open columns) or worker non-nestmates (filled columns) from either monogynous or polygynous colonies. Columns are means (+SE). Sample sizes are listed under the appropriate column; ns, p > 0.05; * p < 0.05; ** p < 0.01; *** p < 0.001.

**Fig. 7**: Proportion of aggression scores between non-nestmates from monogynous (squares) and polygynous (diamonds) colonies. Squares and diamonds are proportions of aggressive scores that fell within the stated range. n = 26 for monogynous colonies; n = 40 for polygynous colonies; * p < 0.01.
Discussion

*P. pallidus* workers from both monogynous and polygynous colonies discriminated against non-nestmates (Fig. 3). However, although workers from polygynous colonies interacted with non-nestmates more frequently (Fig. 2), workers from polygynous colonies were less aggressive to non-nestmates than were workers from monogynous colonies (Fig. 3). The decrease in aggression between non-nestmates from polygynous colonies compared with non-nestmates from monogynous colonies can be attributed to the significantly higher number of acceptance errors (Fig. 7).

The number of interactions and the level of aggression between nestmates from monogynous colonies and nestmates from polygynous colonies were not significantly different (Figs 2 and 3, respectively). However, nestmates from monogynous colonies associated more closely than nestmates from polygynous colonies (Figs 5. 6). Nestmates from polygynous colonies may avoid each other because of the slightly increased likelihood of escalated aggressive interactions (Fig. 7). Our results indicate that, for *P. pallidus*, interactions between non-nestmates and spatial associations between nestmates differ as a function of whether the colony is monogynous or polygynous.

These results are consistent with previous findings. While monogynous ants uniformly reject non-nestmates, polygynous ants often lack any observable behavioral discrimination (reviewed by Fletcher & Michener 1987). Janzen (1973) observed both obligate monogynous and polygynous *Pseudomyrmex* ants (*P. ferruginea* and *P. venenata*, respectively). While workers from the obligate monogynous ant displayed discrimination, the workers from the obligate polygynous ant did not discriminate between nestmates and non-nestmates. As a facultatively polygynous ant, *P. pallidus* appears to be an intermediate in both colony-founding strategies and behavioral repertoires between the obligate monogynous and polygynous species. It appears that the loss of fine-tuned nestmate discrimination behavior is associated with a polygynous founding strategy.

Behavioral differences between monogynous and polygynous colonies in nestmate discrimination are predicted by the optimal acceptance threshold model (Reeve 1989). Reeve’s model predicts that the level of aggression will vary with the level of overlap between nestmate and non-nestmate recognition template-cue dissimilarity distributions (Fig. 1). With an increased overlap between these distributions, the acceptance threshold is predicted to shift to balance the fitness costs of rejecting nestmates and accepting non-nestmates. Thus, should the threshold shift right to become more accepting, the frequency of acceptance errors will increase (Fig. 1).

Our data support this prediction. As the number of queens in a colony increased, aggression between non-nestmates decreased (Fig. 4). The increased number of acceptance errors between non-nestmates from polygynous colonies confirms a threshold shift in polygynous systems relative to monogynous systems (Fig. 7).

A threshold shift may occur due to factors other than increased intracolonial cue diversity (see Starks et al. 1998). For example, because of the lower colony-level relatedness in polygynous relative to monogynous colonies (Peters, unpubl. data), there may be a lower fitness payoff for protecting developing brood in polygynous colonies. However, the pattern of aggression between nestmates from monogynous and polygynous colonies observed suggests that this shift in the acceptance threshold was due to a broader distribution of nestmate recognition cues in polygynous colonies. If the cue distribution for polygynous colonies was not broader than the cue distribution for monogynous colonies, a threshold shift would decrease the observed aggression between nestmates from polygynous colonies relative to monogynous colonies. No such decrease was observed. Thus, our results suggest that workers from polygynous colonies are more likely to share recognition cues with non-nestmates than are workers from monogynous colonies.

The increased overlap between the dissimilarity distributions can result from increased within-colony genetic diversity or from externally derived recognition cues. Since our animals were maintained in similar environments, the likely cause of cue overlap between the nestmates and non-nestmates in polygynous colonies was the increased genetic diversity within these colonies (Peters, unpubl. data).

In conclusion, *P. pallidus* have the ability to recognize and discriminate nestmates from non-nestmates. However, decreased aggressive interactions between non-nestmates and increased distance between nestmates make it apparent as if workers from polygynous *P. pallidus* colonies have a less developed discrimination ability than workers from monogynous colonies. Discrimination errors in polygynous worker interactions suggest that the diversity of nestmate recognition cues is greater in polygynous colonies than in monogynous colonies. These differences in discrimination behavior are consistent with the predictions of Reeve’s optimal acceptance threshold model (1989). We suggest that the optimal acceptance threshold model be of wide application to recognition studies and specifically to studies of ants because of their extreme range of intra- and inter-specific discrimination abilities (Wilson 1971).

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Literature Cited


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