Research article

Thief workers and variation in nestmate recognition behavior in a ponerine ant, *Ectatomma ruidum*

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Abstract. Our findings give new insight into the relationship between nestmate recognition and cleptobiosis, intraspecific thievery of newly collected food items, in a neotropical ponerine ant, *Ectatomma ruidum*. The expression of discrimination of nestmates from non-nestmates varied among local aggregations of *E. ruidum* in a population at Barro Colorado Island, Panama. This result is due to differences in the behavior of the guard ants among aggregations, rather than differences in the expression of recognition cues by ants. Baiting experiments show that *E. ruidum* colonies in Panama have a greater tendency to have overlapping home ranges than a similar population in Costa Rica. The pattern of cleptobiosis, however, is strikingly similar between the Panamanian and Costa Rican populations.

Key words: Nestmate recognition, cleptobiosis, theft, home range, Barro Colorado Island.

Introduction

Intriguing questions concerning nestmate recognition systems in social insects stem from observations of occasional failures of those systems to prevent cleptobiosis or parasitism. Cleptobiotes or parasites often gain entrance to their target colony by chemical mimicry and camouflage (Dettner and Liepert, 1994); they take on the nestmate recognition cues of their target and evade the colony defenders. Breed et al. (1992) and Jeral et al. (1997) documented theft of food between adjacent colonies of *Ectatomma ruidum*, a neotropical ponerine ant and showed that chemical camouflage is involved in this system. In this paper we further explore the role of nestmate recognition in cleptobiosis in this species.

Some *E. ruidum* foragers specialize in patrolling near the nest entrances of other conspecific colonies or enter those colonies (Breed et al., 1990; 1992). These ants, characterized as "thieves" by Breed et al. (1990), engage in cleptobiosis;

as food is brought into the colony they remove the food items and carry them to their own colony. Jeral et al. (1997) showed that thief ants have reduced quantities of cuticular hydrocarbons on their surface and that their cuticular hydrocarbon profile is intermediate between the hydrocarbon profile of their own colony and the colony from which they are stealing. Cuticular hydrocarbons provide recognition cues in many ants (Breed, 1998; Vander Meer and Morel, 1998); Jeral et al.'s (1997) results demonstrate that *E. ruidum* workers rely on a combination of camouflage and chemical mimicry to gain entry into other colonies. An alternative hypothesis to explain the food transfer among colonies is polydomy; multiple nests may be occupied by a single colony and food exchange may be between nests but within colony. The cuticular hydrocarbon data (Jeral et al., 1997) and behavioral data (Breed et al., 1992) from the Costa Rican population support the conclusion of cleptobiosis rather than polydomy.

E. ruidum has a broad geographic distribution, from Mexico to the Amazon, is abundant in many neotropical habitats and has been well studied throughout the Central American portion of its range (Breed et al., 1990; Pratt, 1989; Jaffe and Marquez, 1987; Lachaud, 1985; 1990; Perfecto and Vandermeer, 1993). Where the species is present, colonies are often very dense, up to 0.75 per square m (Levings and Franks, 1982; Breed et al., 1990). While no formal characterization has been made of its preferred habitats, it appears to be characteristic of leaf litter communities in tropical moist and tropical dry forests, and is found in disturbed areas of tropical wet forests where the ground layer is exposed to sunlight.

E. ruidum nests in small subterranean colonies, usually with less than 250 workers, and colonies are often monogynous. The prevalence of single-queened colonies (Pratt, 1989; Breed et al., 1990), the expression of nestmate recognition by workers (Breed et al., 1990; 1992), territorial interactions among foragers (Breed et al., 1990), and biochemical differentiation among colonies (Jeral et al., 1997)

all support the argument that colonies in populations of this species are discrete, monodomous social entities.

In addition to raising questions about nestmate recognition, the presence of intraspecific cleptobiosis in the Costa Rican population of *E. ruidum* studied by Breed et al. (1990, 1992) and Jeral et al. (1997) stimulates inquiry about the generality of such behavior in this species. Among other published observations of foraging and other behavior of *E. ruidum* in Mexico (Lachaud, 1985, 1990, 1996; Schatz et al., 1995; De Carli et al., 1998), Nicaragua (Perfecto and Vandermeer, 1993), Panama (Pratt, 1989), and Venezuela (Jaffe and Marquez, 1987), intraspecific cleptobiosis has only been recorded in the Mexican population.

We determined if the relationship between the nestmate recognition system of *E. ruidum* and the presence of cleptobiosis is consistent within a larger population of this species than occurred at our study site in Costa Rica. We then explored how colony foraging home range and cleptobiosis might interact. Finally, as our experiments were conducted on Barro Colorado Island, Panama, we use our data to compare patterns of cleptobiosis between populations of *E. ruidum*.

Methods

During the dry season, 1998, we located colonies of *Ectatomma ruidum* on Barro Colorado Island (BCI), Panama. Colonies were mapped in four plots near trails: 1) the laboratory/residential clearing, 2) 225 m from the station buildings on the Snyder-Molino trail, 3) 25 m off the Snyder-Molino trail on the Barbour trail, and 4) 13–25 m from the Snyder-Molino trail on the Wheeler trail. Locations 2–4 are in mature secondary forest with limited undergrowth, a forest type that is typical of the southeastern portion of the island. Plot size was constrained by variations in topography, such as steep hills. Nests were located by presenting foraging ants with small amounts of food. This stimulated the ant to return to its colony; nests and foraging sites were marked with flagging. Each site was used repeatedly for experiments, so that the rate of discovery of new nests declined to zero. The nest maps for each area therefore represent exhaustive samples (Breed et al., 1992).

Nest densities varied from 0.12 per square m at site three (11 colonies, 88.5 square m) to 0.58 per square m at site one (10 colonies, 5.75 square m). At site 2 the density was 0.42 per square m (42 colonies, 100 square m) and at site 4 it was 0.14 per square m (21 colonies, 150 square m). These densities are consistent with densities reported at BCI by Levings and Franks (1982) and at La Selva by Breed et al. (1990). At sites three and four, *Odontomachus* spp. were commonly observed in predation attempts on *E. ruidum*. *E. ruidum* nests were absent from areas of high *Odontomachus* concentration.

Experiment one: are there thief ants in this population? Thief ants are best discovered by presenting food *ad lib*. at nest entrances. We used yellow cheese as bait; this food does not occur naturally and its color allows for easy identification of stolen food items. Foragers collect the food and take it into the nest; thieves, if present, can then be observed leaving the colony with stolen food items. After presentation of the bait, nests were observed for ten min and the nests to which the food was carried by the thieves were recorded.

Experiment two: is there evidence for nestmate recognition? Nest entrances were observed for 20 min. The number of thieves leaving with naturally occurring food items and the number of fights between nest residents and intruding ants were recorded. Agonistic behavior in this species is described in detail by Breed et al. (1990). Bait (yellow cheese) was then presented at each nest entrance and another 20 min of observation ensued, with the same variables recorded. The first observation period obtains the normal rate of thievery and fighting in this

population, while the presentation of the bait for the second observation period was designed to stimulate both thieving and fighting, giving a measure of the potential for these behavioral patterns in the population.

Experiment three: do transfers among nests indicate spatial variation in nestmate recognition cues? Following the methods of Breed et al. (1992) the acceptability of ants transferred from one nest to another was tested. These tests allowed us to determine if the nests within a site were unicolonial (all extensions of the same colony, and consequently mutually compatible) or if each nest represents a single colony as evidenced by mutual incompatibility.

Foragers were presented with bait and followed to their nest, where they were collected as they entered. Each forager was then placed in a clean 25 ml glass scintillation vial, and chilled to immobility by placing the vial on ice. Once immobile, the forager was removed from the vial with forceps and placed on either its own nest entrance (controls) or another nest entrance (experimentals). Experimental transfers were done both within and between sites. Within site transfers were conducted haphazardly with regard to the spatial relationship of source and recipient nest, except at site four, where a more comprehensive picture of intercolonial relations was constructed by testing all possible colony combinations in a 25 square m subplot housing six colonies. The number of colonies used at each site is indicated in Table 2. Each recipient colony was used an equal number of times, although some tests were excluded from the final results if there were no encounters between the test ant and the resident ants; this was partcularly a problem at site 4.

The immobile ants were observed until a behavioral response was elicited from the ants in the recipient nest. The most common responses of the resident ants were to ignore the intruder, which we scored as an acceptance of the donor ant, or to drag the intruder away from the nest entrance, which we scored as a rejection by the resident ants. Breed et al. (1992) gives a more detailed account of the behavioral responses, which did not differ between the Costa Rican and Panamanian populations.

Experiment four: are nest foraging ranges indicative of territoriality? We wanted to determine if the foraging range of this species was consistent with the non-overlapping home ranges that Breed et al. (1990) observed in a Costa Rican population, or if ranges were broadly overlapping, as might be predicted if the nests in this population are unicolonial. Site two was topographically appropriate for this test and had a sufficient number of colonies for a meaningful analysis. A 5 m by 5 m grid was laid out with string and bait was placed at each 1 m grid intersection. As ants discovered the baits, the location of the bait and the nests to which the ants returned were noted.

Results

Experiments one and two: are there thief ants and is there evidence for nestmate recognition in this population? Prior to baiting in experiment two, a mean of 0.019 (SE = 0.005, n = 40 colonies) thieves per minute were observed leaving colonies with food items. A mean of 0.23 (SE = 0.005) thieves left colonies per minute after *ad lib*. baiting in experiment one (n = 33 colonies). Each robbed colony in experiment one was targeted by a mean of 1.4 (SE = 0.17, n = 31, range = 1 to 3) other colonies. Robbing colonies were typically the nearest neighbors of the colony being robbed.

In control observations 0.02 (SE = 0.006) agonistic encounters per min were observed. The mean rate of agonistic encounters increased to 0.03 (SE = 0.007) per min after baiting and the rate of thievery increased to 0.20 (SE = 0.028) per min, although some incidents of thievery involved removal of food from the immediate area of the nest entrance, rather than from inside the nest. The difference between before and after bait presentation in the rate of agonistic encounters was not significant (Wilcoxon paired

Site	Agonistic acts before bait	Agonistic acts after bait	Thieves before bait	Thieves after bait	N
1	0.03 ± 0.013	0.06 ± 0.018	0.01 ± 0.006	0.18 ± 0.058	8
2	0.01 ± 0.008	0.04 ± 0.024	0.00 ± 0.000	0.28 ± 0.081	8
3	0.01 ± 0.008	0.00 ± 0.000	0.04 ± 0.012	0.18 ± 0.067	8
4	0.02 ± 0.011	0.01 ± 0.007	0.02 ± 0.007	0.18 ± 0.039	16
	H = 1.86, ns	H = 9.56, p = 0.02	H = 8.83, p = 0.03	H = 1.60, ns	

Table 1. Between site comparison of agonism and thievery rates, converted to the number of acts observed per minute, given with results of the Kruskall-Wallis non-parametric test between site for each variable. Means are presented with their standard errors

ranks test, $Z = 1.00$, $p = 0.32$), but the difference in rate of thievery was significant (Wilcoxon paired ranks test, $Z = 4.86$, $p < 0.0001$).

Additional insight is gained by comparing the rates of agonism and thievery among the four sites (Table 1). The rate of agonism varies among site after bait presentation, but before. Sites 1 and 2 have a much higher rate of agonism than do sites 3 and 4. The number of thieves observed prior to baiting varied significantly among sites, but there was no significant variation after baiting.

Experiment three: do transfers among nests indicate spatial variation in nestmate recognition cues? Table 2 shows the results from within site transfers of worker ants from one colony entrance to another. Incompatibility among colonies was observed at all four sites, but there was significant ($\chi^2 = 9.50$, df = 3, p = 0.02) heterogeneity among the sites in the frequency of rejections of non-nestmates; site four had a much higher rate of internidal compatibility than the other three sites; this is consistent with the result from experiment two, in which agonism occurred in lower frequency at site 4. However, at site four we noted marked variation among colonies in their responses to introduced ants ($\chi^2 = 20.0$, df = 9, p = 0.018). At site one there was no significant heterogeneity among the response of the five nests used as recipients ($\chi^2 = 1.25$, df = 4, ns). Similar patterns held at site 2 (recipients, $\chi^2 = 12.76$, df = 11, ns) and site 3 (recipients, $\chi^2 = 0.00$, df = 7, ns).

Our finding in site 4 led us to explore internidal relationships by completing an interaction matrix (Table 3) for the site. Excluding the controls, the overall result from this experiment at site 4 is not significantly different ($\chi^2 = 0.61$, df = 1, ns) from the overall result from the experiment shown in Table 2, which involved an overlapping but different set of colonies. Colonies at this site did not significantly vary in their acceptance of foreign workers ($\chi^2 = 7.78$, df = 5, ns) but did in their worker's acceptability to other colonies ($\chi^2 = 11.13$, df = 5, p = 0.049). Colonies U and T were more likely, but not statistically significantly so, to reject foreign ants, and ants from colonies O, L and T were more likely to be rejected when introduced. Colonies O, L, and T were slight outliers with respect to the distribution of the rest of the colonies, which were clustered near the center of the plot. Of all the colonies, O and T were the only entirely mutually incompatible pair in this assay.

In addition to compatibility tests within site, we tested ants from site 1 at site 4 and *vice versa*. 13 of 20 ants from

Table 2. Acceptance or rejection of chilled ants by resident ants in nests at each site. Chilled ants were foragers obtained from other nests at the same site.

	Site 1	Site 2	Site 3	Site 4
Rejected	13	16	15	9
Accepted	4	5	4	13
N colonies tested	9	12	8	16

site four were rejected by site one colonies, and 8 of 21 ants from site one were rejected by site four colonies. Adding the data from Table 2 for sites 1 and 4 to the data for this experiment yields a significant difference between recipient sites ($\chi^2 = 7.56$, df = 1, p = 0.006) on whether introduced ants are accepted by colonies. However, when the data are analyzed to determine if there is an effect of the source of the ant, no significant difference was present ($\chi^2 = 0.07$, df = 1, p = 0.77). Site one ants were much more likely to be rejected by colonies other than their own at site one than by colonies at site four ($\chi^2 = 5.60$, df = 1, p = 0.02), further supporting the suggestion that it is the behavior of the ants in the recipient colony, rather than the characteristics of the donor ants, that determines the outcome.

Experiment four: are nest foraging ranges indicative of territoriality? 173 ants were attracted to the baits in the grids and were followed to their nests. The foraging pattern for

Table 3. Internidal compatibility for site 4. The recipient column indicates the reaction of the colony when ants from other colonies were introduced into it. The donor column shows how other colonies reacted to ants from that colony. The controls are introductions of foragers to their own colony. The number on the left of the slash in each cell is the number of rejections, to the right of the slash is the number of acceptances.

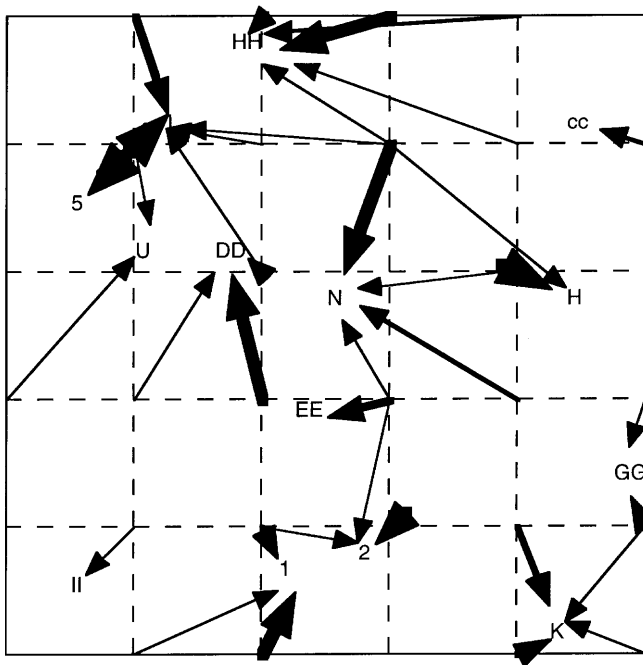| Colony	Recipient	Donor	Control
R	2/8	2/8	1/1
U	6/4	1/9	0/2
O	2/8	5/5	0/2
T	5/5	6/4	0/2
K	2/8	1/9	0/2
L	2/8	4/6	0/2
Total	19/41	19/41	1/11

Figure 1. Foraging patterns revealed by baiting at site 2. The letters and numbers correspond to nest locations. Baits were placed at the grid intersections and the number of ants discovering each bait and transporting food to a nest were recorded. The width of each arrow is proportional to the number of ants travelling from an intersection to a given nest; the narrowest arrrows represent one ant, the widest five or more ants

134 ants at site 2 is shown in Figure 1; foraging home ranges are clearly non-overlapping, although some baits at home range boundaries were shared among two or more colonies. No agonism was observed at shared baits, as effort was concentrated on food collection rather than defense.

Discussion

Our most striking finding is the difference among local aggregations of *E. ruidum* colonies in their expression of discrimination between nestmates and non-nestmates. However, even at those sites where we found a high level of nestmate discrimination, conspecific cleptobiosis was common. The general pattern of cleptobiosis conforms to our previous observations in Costa Rica. In the Panamanian population our test of the polydomy hypothesis relies on the assumption that nestmate recognition is an expression of colonial differences; the existence of nestmate recognition among neighboring colonies is supportive of the cleptobiosis hypothesis for the Panamanian population.

The diminution of discrimination between pairs of colonies at site 4 may be explained by either genetic similarity among colonies or a local reduction in colonial vigilance. Genetic similarity among adjacent colonies could result from reproduction by budding, fission or by queens settling near their natal nest, resulting in high population viscosity. If any

of these factors play a role in the population, then genetically determined recognition cues would be more similar among adjacent colonies than in the population as a whole. Explanatory hypotheses for reduced vigilance include food not being a limiting factor for colonies and the presence of other threats to colonies that distract defenders from conspecific cleptoparasites.

An analysis of our data favors the hypothesis of reduced vigilance. The transfers of ants between sites 1 and 4 show that the lower level of expression of discrimination is not because ants from site 4 lack recognition cues – they are discriminated at site 1 – but is due to reduced vigilance at the site 4 colonies, which are as accepting of site 1 ants as site 4 ants. This finding argues against genetic similarity among nearby colonies as an explanation for the behavioral differences between sites 1–3 and site 4. This finding is consistent with Jeral et al.'s (1997) finding of biochemical differences between ants from adjacent nests. The issue of genetic similarity among colonies awaits genetic studies of *E. ruidum* populations for a final resolution.

It is particularly significant that thievery continues at the same rate over a four-fold range in colony density, from 0.12 per square m to 0.58 per square m. This is a factor that we were unable to test at La Selva and indicates that expression of the behavior is not dependent on maximal colony densities.

The populations of *E. ruidum* at Barro Colorado Island and La Selva are quite similar in the pattern of thievery among colonies. This finding supports the conclusion that the behavior seen in the La Selva population (Breed et al., 1990, 1992; Jeral et al., 1997) is not an aberration or pathology due to the disturbed nature of the habitats in which *E. ruidum* occurs at La Selva. The presence of a nestmate recognition system that allows discrimination of ants between nearby colonies is consistent with our (Breed et al., 1990) argument that *E. ruidum* populations consist of independent monodomous colonies, rather than being unicolonial. As at La Selva colonies at BCI have non-overlapping foraging home-ranges, another indicator of monodomy. Even though there is local variation in the expression of nestmate recognition, as at our fourth site in this study, in general thievery persists in *E. ruidum* populations despite the presence of nestmate recognition. The span between La Selva and BCI is small relative to the range of this species in the Neotropics, and studies of other populations may give more insight into phenotypic variability of behavior in widespread species.

Acknowledgements

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