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Mechanism of template-label matching in fire ant, *Solenopsis invicta* Buren, nestmate recognition

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Abstract. Aggression bioassays were used to investigate how fire ant workers 'match' perceived odour labels of encountered individuals with a learned template of nestmate odours. A split-colony design was used, with parent colonies and subcolonies receiving different diets. Diets either shared all components, shared some components but contained no unshared components, or contained both shared and unshared components. Results suggest that workers 'compared their colony odour template with labels of encountered kin by 'overall similarity' rather than by a 'discrete odour' mechanism requiring either (1) exact cue correspondence between templates and labels, (2) rejection of an individual whose label contains any cue not present in the template (foreign label rejection), or (3) acceptance of an individual whose label contains any cue present in the template (habituated label acceptance).

Discrimination of colony members from nonmembers on the basis of odour differences is a widespread phenomenon in social insects (Wilson 1971; Hölldobler & Michener 1980; Fletcher & Michener 1987). This discrimination is typically based on 'phenotype matching' (Holmes & Sherman 1983) in social Hymenoptera (Buckle & Greenberg 1981; Pfennig et al. 1983; Carlin & Hölldobler 1986). During phenotype matching, colony members compare olfactory attributes or 'labels' of encountered individuals with a memory 'template(s)' of odours possessed by self, nestmates or nest substrate (see also Alexander 1979; Lacy & Sherman 1983; Sherman & Holmes 1985). Decision rules by which animals compare labels and templates are for the most part unaddressed by empirical studies, although several mechanisms have been proposed (Crozier & Dix 1979; Getz 1982; Getz & Chapman 1987). Getz (1982) suggested three mechanisms of template-label matching with respect to heritable odour cues mediating hymenopteran kin recognition. These are: (1) accept an encountered individual if all cues in that individual's label are present in the template (genotype matching of the original model, referred to hereafter as exact label acceptance), (2) reject an individual if its label contains any cue not present in the template (foreign label rejection), and (3) accept an individual if its label contains any cue present in the template (habituated (common) label acceptance).

Getz's original models posited that labels and templates comprise a limited number of discrete and individually recognizable odours. This assumption is not required in more recently proposed 'cue similarity' models (Gamboa et al. 1986a, b; Getz & Chapman 1987), in which acceptance or tolerance is based upon an individual perceiving that the incoming odour label is sufficiently similar to its template of 'odour images' (Getz & Chapman 1987). In the model of Getz & Chapman (1987), the similarity between perceived and template odours is based on neural processing attributes of insects and can be represented by a scaler quantity. In addition, both models postulate a threshold (i.e. non-graded) response to cue similarity, i.e. increasing similarity between the learned and the perceived cue does not result in increasing tolerance. Extensive empirical evidence supports the threshold response hypothesis for the primitively social wasp *Polistes fuscatus* (Gamboa et al. 1986a, 1987; Gamboa 1988).

The present study investigates template matching decision rules in monogyne laboratory colonies of the imported fire ant, *Solenopsis invicta* Buren (Myrmicinae). Under laboratory conditions, diet-derived cues can dominate the colony odour of this species (Obin 1986, 1987; Obin & Vander Meer 1988). We therefore tested recognition between kin groups reared on diets that either contained identi-

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cal components, foreign components, or common components. Results would suggest how olfactory cues were organized in templates and how these templates were matched to incoming odour labels of encountered individuals.

MATERIALS AND METHODS

Ant Rearing

Monogyne *S. invicta* colonies were established in the laboratory from newly mated queens collected near Gainesville, Florida (see Banks et al. 1981, for details of collecting and initial queen maintenance). At maturity, these mature 'resident' colonies each contained 60000–100000 workers, a physogastric (highly fecund) queen, and all brood stages. Colonies were housed in petri dish nests with Castone floors, and foraged in plastic trays (52·0 × 39·0 × 7·5 cm; Obin 1986). Queenless subcolonies containing 200 workers of each of the three temporal subcastes (i.e. nurses, reserves and foragers; Mirenda & Vinson 1981) and 500–600 larvae were established from each mature colony (see below). These queenless 'intruder' groups were maintained in individual metal pans (5·5 × 17·5 × 29·0 cm) equipped with a petri dish nest (with Castone floor) and a cotton-stoppered water tube. Rearing room temperatures ranged from 21 to 24°C, and the light–dark cycle was variable.

Fire ants require a diet containing carbohydrate, lipid and protein. Accordingly, diets included some or all of the following material (see below): 50% (v:v) clover honey in water, 50% (v:v) dark cane syrup in water, equal parts honey-water and syrup solutions, 5% (w:v) granulated sugar, solution, thawed moth pupae, *Anticarsia gemmatalis*, thawed roaches, *Periplaneta americana*, and live meal worms. Solutions were stored at 4°C, and moth pupae and roaches were stored at −16°C. Colonies were fed ad libitum, and food dishes were replenished every 24–36 h.

Dietary odours may be adsorbed directly into the integument or modified metabolically after ingestion. This study assumed that (1) metabolically produced odour cues of workers were identical unless colonies contain unshared diet elements, and that (2) no direct or metabolically correlated unique olfactory cues resulted from dietary sucrose. Both assumptions rule out any asymmetric effects of microorganisms.

Experiment 1

Three subcolonies were established from each of 12 queenright, resident colonies, and diet treatments were begun. Queenright resident colonies received honey-water and moth pupae. The three subcolonies received either sugar solution and roaches, honey-water and roaches, or the resident colony diet of honey-water and moth pupae. After 1 month, three intruders from each of the three queenless subcolonies were introduced into the foraging arena of their parent resident colony, and subsequent aggression was quantified (see Bioassay). The sequence of intruder types presented to any colony was alternated between replications, with no colony receiving an additional intruder until all colonies had been tested an equal number of times. A replicate series of tests was conducted 1 week later. Thus, 216 ($12 \times 9 \times 2$) introductions were performed.

Experiment 2

Four subcolonies were established from each of 11 resident colonies, and diet treatments were initiated. Resident colonies received honey-water and equal parts by weight moth pupae and roaches. Subcolony diets included sugar solution and roaches, honey-water and roaches, honey-water and moth pupae and the resident colony diet (honey-water, moth pupae and roaches). After 6 weeks, four intruders from each subcolony were introduced in alternating sequence (as above) into the resident colony ($N = 11 \times 4 \times 4 = 176$ introductions).

Experiment 3

Nine colonies tested in experiment 2 and three of each of their subcolonies were used in this experiment. Mealworms were added to resident colony diets, and honey-water was replaced with the honey-water/cane syrup mixture. Subcolonies were fed identical insect material as parent colonies, but received either 50% honey-water, 50% cane syrup or the honey/syrup mixture. Four months after diets were established, three workers from each subcolony were introduced in alternating sequence into their parent colony. A replicate series of tests was conducted 1 week later ($N = 9 \times 9 \times 2 = 162$ introductions).

When trials required more than 1 day to complete, each colony was tested at the same time of day (±0·5 h) throughout the experiment.

Table I. Behavioural units and aggression scores (1–9) used in nestmate recognition bioassay

Rank	Behaviour directed toward intruder
1	Intruder antennated for less than 2 s; if mobile, is not followed; if intruder is stationary, resident ant does not stop
2	Intruder antennated (as in 1); if mobile, intruder is followed slowly for several centimetres; if intruder is stationary, resident stops
3	Rapid antennation of intruder, antennae extended for more than 2 s
4	Mandible gaping; rapid antennation; 'sidling' (maintaining a lateral orientation to and slowly circling intruder)
5	Alarm (running, abdomen elevation and vibration) and recruitment
6	Intruder 'held' in mandibles by petiole or appendages, but released; biting
7	Intruder held (as in 6), but released; abdomen-curling (stinging posture) by residents, but no stinging; biting
8	Intruder surrounded and held in mandibles by petiole and appendages; appendages pulled/bitten off; eventual stinging
9	Immediate lunge, grab and stinging

Nestmate Recognition Bioassay and Data Analysis

The recognition bioassay measured agonism in the context of nest defence. Individual workers from subcolonies (intruders) were allowed to walk undisturbed onto a pair of extended forceps and were then introduced into their queenright parent colony. Only ants that walked undisturbed from the forceps into the parent colony (residents) were tested. Intruder ants were never held in the forceps. Intruders were positioned so as to maximize the initial distance between the site of introduction and resident ants as well as the distance from any previous introduction. Observations were made from a distance of 15 cm, and the observer (M.O.) wore a particle mask to minimize the agitation-inducing effects of exhalation on the ants. Individuals were tested once and were removed from resident colonies after 20 encounters. Experiments were not conducted 'blind' with respect to diet treatments. The ants scatter readily recognizable pieces of diet throughout the rearing tray. Removing this material before testing alarms the entire colony. Staging encounters in clean, neutral arenas was also not feasible, since fire ants interact minimally in such a context.

Colony response to each intruder was scored on a (1–9) scale of increasing aggressive behaviour (Table I). As a precaution against possible effects of non-independent behavioural units, subsequent statistical tests (Sokal & Rohlf 1981) were based on the single most aggressive response observed during an intruder's interaction with 20 resident ants. First, mean ($\pm$SD) responses for each treatment were computed from individual introduction scores (Table II). However, due to non-normal distribution of the data, overall treatment effects were assessed with the (non-parametric) Kruskal-Wallis test. Subsequent designations of significantly different pairs of treatment means (Table II) were based on consensus between Tukey's -w method (parametric) and a simultaneous test procedure employing the Wilcoxon statistic obtained for each paired comparison (non-parametric). The experiment-wise error rate for both multiple comparison tests was set at 0·05. Due to potential circadian and seasonal variation in recognition response, we avoided statistical comparisons involving results of different experiments.

RESULTS

Experiment 1

Data (Table IIA) indicate a significant treatment effect of diet ($H=17{\cdot}47$, $df=2$, $P<0{\cdot}001$). Intruders fed either sugar and roach diet or honey and roach diet elicited significantly more aggression from resident colonies than intruders maintained on the honey and moth pupae diet shared with resident colonies. Aggression directed at intruders fed either sugar and roach or honey and roach diets was not significantly different ($P>0{\cdot}05$).

Experiment 2

There was a significant effect of diet treatments ($H=18{\cdot}00$, $df=3$, $P<0{\cdot}001$; Table IIB), with significantly more aggression directed at intruders maintained on the sugar and roach diet than at all other treatment groups. The magnitudes of aggression directed at intruders from the three other diet groups were not significantly different ($P>0{\cdot}05$).

Experiment 3

There was a significant effect of diet treatments ($H=12{\cdot}29$, $df=2$, $P<0{\cdot}001$; Table IIIC). Signifi-

Table II. Mean (±SD) aggression elicited from resident colony workers by kin intruders fed diets either identical to the resident colony diet, containing some components of the resident colony diet but no unique components, or containing both common and unique components

Resident diet	Intruder diet (*N*)	Resident response
(A) Honey + pupae	Honey + pupae (72)	$3{\cdot}25 \pm 2{\cdot}12^{a}$
	Honey + roach (72)	$4{\cdot}07 \pm 2{\cdot}42^{b}$
	Sugar − roach (72)	$4{\cdot}50 \pm 2{\cdot}38^{b}$
(B) Honey + pupae + roach	Honey + pupae + roach (44)	$3{\cdot}30 \pm 2{\cdot}08^{a}$
	Honey + pupae (44)	$3{\cdot}55 \pm 1{\cdot}98^{a}$
	Honey + roach (44)	$3{\cdot}41 \pm 2{\cdot}28^{a}$
	Sugar + roach (44)	$5{\cdot}11 \pm 1{\cdot}87^{b}$
(C) Honey syrup + mealworms + pupae + roach	Honey/syrup + mealworms + pupae + roach (54)	$2{\cdot}76 \pm 1{\cdot}37^{a}$
	Honey + mealworms − pupae − roach (54)	$3{\cdot}54 \pm 1{\cdot}65^{b}$
	Syrup + mealworms − pupae − roach (54)	$3{\cdot}83 \pm 1{\cdot}67^{b}$

Means with the same superscript were determined to be not significantly different by both parametric and non-parametric multiple comparison tests (experiment-wise error rate = 0·05). *N*: number of individual intruders tested.

cantly more aggression was directed at intruders fed either diet lacking a component (either honey or cane syrup) of the resident colony diet. The magnitudes of aggression directed at intruders from these two diet groups were not significantly different ($P > 0{\cdot}05$).

DISCUSSION

This study addressed how fire ant workers compare memory-based odour templates with the incoming odour labels of conspecifics, and is to our knowledge, the first empirical study designed specifically to test the odour-matching mechanisms proposed by Getz (1982). Since only kin were tested against each other, and since transferred queen discriminators appear to play no role in our fire ant nestmate discrimination assay (Obin 1987; Obin & Vander Meer 1988, 1989), discordance between resident templates and intruder labels was due solely to differences in diet-derived odours. In reality, heritable worker discriminators as well as other environmentally acquired cues also contribute to differences in colony labels (Obin 1986, 1987; Obin & Vander Meer 1988). Accordingly, the results of this study cannot be generalized to the field situation at the present time.

Acceptance under an 'exact cue acceptance' model of template matching requires that all diet components be represented in the intruder label. Although results of experiments 1 and 3 appear to support this mechanism, intruders from the honey and moth pupae and honey and roach diet groups in experiment 2 were treated no differently by resident colonies than intruders maintained on the complete resident colony diet of honey, moth pupae and roaches. A mechanism involving 'foreign-label rejection' requires that any component not represented in the resident colony diet elicit aggression. While the results of experiment 1 cannot rule out this mechanism (roach is a foreign component), the results of experiments 2 and 3 conclusively exclude it. Under our assumption that sucrose solution provides no olfactory recognition cues, the sugar and roach diet that elicited strong aggression in experiment 2 has no foreign cues. More convincing perhaps are the results of experiment 3, in which foreign cues were unequivocally absent from any subcolony diet, yet two of the diets elicited increased aggression. Finally, intruders were attacked despite the fact that their diets shared common elements with resident diets (experiments 1, 2 and 3). This result argues against a recognition mechanism involving 'habituated label acceptance'.

Data for fire ants therefore support only an 'overall similarity' model of template-label matching (Gamboa et al. 1986a, b, 1987; Getz & Chapman 1987; Gamboa 1988). Our results also suggest that 'similarity' may depend on the total number and relative contribution of colony odour cues to worker labels and templates. When differences in labels were derived from two-component diets (experiment 1), odour cues from both components of the resident diet were necessary for complete concordance between labels and templates. With a three component diet (experiment 2), only two shared components were necessary for a functional match between label and template. In experiment 3, odour cues derived from honey and syrup appeared to dominate the template such that, despite four shared diet components, intruder labels required both honey and syrup in order to match worker templates. Although no experiments were designed expressly to evaluate whether the perception of cue similarity was a threshold or non-threshold phenomenon, multicomponent diet experiments similar to those presented here seem particularly suited to such studies.

Finally, empirical distinctions between any of the discrete odour-matching models may in fact, be epiphenomena. For example, recognition studies of the carpenter ant, *Camponotus*, spp., have been interpreted as evidence for both 'genotype matching' (Morel et al. 1988) and 'foreign-label rejection' (Carlin & Hölldobler 1987; Morel, unpublished data). While animals may use different matching mechanisms in different behavioural and experimental contexts, the parsimonious explanation of the *Camponotus* data entails 'cue similarity'. Discrete odour models may also blur important distinctions with respect to mechanisms of label acquisition. Consider the oft-invoked 'gestalt' mechanism (Crozier & Dix 1979) in which olfactory cues are transferred among workers, thereby producing a colony odour blend shared by all nestmates. Attempts to demonstrate a colony odour 'gestalt' typically posit a graded recognition response as a function of incremental changes in the proportion of kin in mixed groups (e.g. Carlin & Hölldobler 1986). As formulated, none of the discrete odour models is germane to this approach, whereas a non-threshold 'cue similarity' model is entirely appropriate. Failure to obtain a graded response may be evidence in favour of a threshold matching mechanism, not evidence against a colony odour blend. Our efforts to comprehend more fully recognition by phenotype matching will be well-served by additional studies of template and label formation in animals.

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