Sources of nestmate recognition cues in the imported fire ant
Solenopsis invicta Buren (Hymenoptera: Formicidae)

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Abstract. Aggression bioassays were conducted to investigate nestmate (worker–worker) recognition in monogyne colonies of the imported fire ant. Environmentally correlated cues dominated the recognition cue hierarchy of laboratory and field colonies, and diet alone significantly modified recognition labels and templates of laboratory-reared workers. 'Discriminators' associated with worker genotype also affected recognition, but 'queen discriminators' did not significantly affect either labels or templates of laboratory-reared workers exposed as adults to alien queens for 28 days. Factors contributing to the importance of environmentally derived recognition cues in this species and the potential implications of such cues for the formation of polygynous fire ant populations are discussed.

The ability to discriminate colony members or nestmates from non-nestmates (i.e. nestmate recognition) is essential for the stability of insect societies in both ecological and evolutionary time (Wilson 1971). As in recognition systems of many invertebrate and vertebrate species (reviewed in Hepper 1986), nestmate recognition in social insects is mediated by olfactory cues (Hölldobler & Michener 1980; Bradshaw & Howse 1984). These chemical recognition cues can be environmentally determined or acquired (Kalmus & Ribbands 1952; Lange 1960; Renner 1960; Free 1961, cited in Wilson 1971; Jutsum et al. 1979; Breed 1983; Stuart 1985; Gamboa et al. 1986a) as well as genetically correlated (Kukuk et al. 1977; Greenberg 1979; Mintzer 1982; Mintzer & Vinson 1985). In theory, genetically correlated recognition cues or 'discriminators' (Hölldobler & Michener 1980) can be produced by both workers and queens of social Hymenoptera (Crozier & Dix 1979; Hölldobler & Michener 1980). To function as nestmate recognition cues, queen discriminators must be transferred to workers. These transferred discriminators are thus analogous to the maternal odours used for sibling recognition by certain vertebrates (Porter et al. 1981; Waldman 1981). To date, transferrable queen discriminators have been demonstrated for small laboratory colonies of the ants *Camponotus*

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spp. (Carlin & Hölldobler 1983, 1986, 1987; Carlin et al. 1987), *Myrmica* spp. (Brian 1986) and *Leptothorax lichtensteini* (Provost 1987, but see Stuart 1985), and suggested for the honey bee, *Apis mellifera* (Breed 1981, 1987; Boch & Morse 1982).

Here we explore the source(s) of cues mediating worker–worker recognition in monogyne (single queen) colonies of the imported fire ant. Using agonism in the context of nest defence as an index of recognition, we first measured the recognition response between former nestmates reared in different environments (laboratory or field). We subsequently evaluated the effect of diet alone on nestmate recognition in small (less than 200 workers), queenless colonies and in larger (more than 10 000 workers), queenright colonies. We then investigated the individual effects of alien queen and alien worker presence on recognition between nestmates and non-nestmates in small, laboratory colonies.

Previous studies (Obin 1986, 1987) and evidence obtained for other social insects (reviews in Gadagkar 1985; Breed & Bennett 1987), suggest that *S. invicta* workers discriminate nestmates from non-nestmates by matching phenotypic 'recognition labels' expressed by encountered individuals with a 'template' (Alexander 1979) of learned, nestmate-borne or self-borne cues ('phenotype matching' of Holmes & Sherman 1982; see also Beecher 1982; Blaustein 1983). By performing reciprocal tests between treatment groups in each experiment, we

were able to assess the effects of treatments on both worker recognition labels and recognition templates.

METHODS

Behavioural Assay and Data Analysis

The recognition bioassay quantified the behavioural response of workers to individually introduced, 'intruder' conspecifics. Introduced ants were intermediate-sized (media) workers of the 'reserve' temporal subcaste (Mirenda & Vinson 1981), and were selected from the area of the rearing tray immediately surrounding the brood cell (laboratory colonies, experiments 1 and 2) or from immediately beneath the mound tumulus (field colonies, experiment 1; laboratory colonies, experiment 3). Intruder ants were allowed to walk undisturbed onto a pair of extended forceps. Only ants that subsequently walked undisturbed from the forceps into the 'resident' colony were tested. Intruder ants were never held in the forceps. Intruders were positioned so as to maximize both the initial distance (5–20 cm) between the site of introduction and resident ants as well as the distance from any previous introduction. The forceps were rinsed with hexane and air-dried after each introduction. Ant behaviour was observed with 10× magnifying glasses. In addition, the observer (M. O.) wore a particle mask to minimize the agitation-inducing effects of exhalation on the ants. Some tests required more than 1 day to complete. In such cases, each colony was tested at the same time of day (± 0.5 h) throughout the experiment.

Colony response to intruders was scored on a numerical scale (1–9) of increasing, aggressive behaviour (Table I). We recorded the most aggressive response observed during an intruder's interaction with 20 resident ants. (The use of only a single datum was a precaution against non-independence of behavioural units.) Introduced ants eliciting behaviours of ranks 1–3 are, at most, 'investigated'; those eliciting behaviours of ranks 4–7 are 'challenged' or repulsed, but they continue to move about the colony; intruders eliciting behaviours of ranks 8–9 are always killed. Mean ($\pm$ SD) recognition responses for treatments were calculated from individual introduction scores. However, because data exhibited intractable, non-normal distributions, non-parametric methods (Wilcoxon two-

Table I. Behavioural units and aggression scores (ranks) used in fire ant nestmate recognition bioassays

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

sample test, two-tailed) were used for tests of significance. Attained significance levels (P) were based on the distribution of the standard deviate (t) computed from the mean and standard deviation of the Wilcoxon rank sum (T) (Sokal & Rohlf 1981).

Experiment 1

This experiment tested reciprocal recognition between workers removed from field colonies and maintained in the laboratory and workers from the field colony of origin. Worker ants ($N=400$–600) and brood were collected from 15 individual, monogyne field colonies and re-established with original nest soil in the laboratory. Ants were maintained at 26–27°C under variable, fluorescent illumination in metal pans ($5.5 \times 17.5 \times 29.0$ cm) equipped with a petri dish brood cell and a cotton-stoppered water tube. A honey-water and roach, *Periplaneta americana*, diet was provided on the third day after collection and every third day thereafter until the completion of the experiment.

The aggressive response of laboratory-maintained subcolonies toward individual workers ($N=2$) introduced from the field colony of origin and from their own subcolony was measured at 2

days and 27 days post-collection. Comparisons were therefore based on 30 (2 × 15) replicates per treatment. The sequence of intruder types was alternated. We also measured the aggressive response of each field colony to former nestmates maintained in the laboratory and to field colony nestmates. In this procedure, a Castone-filled petri dish was baited with a roach and placed approximately 35 cm from the nest mound. Following discovery and recruitment to the arena by workers, alarm was generated by placing the crushed head of a worker (source of alarm pheromone; see Wilson 1962) in the arena. After 1 min, an intruder was introduced into the arena and behavioural data were collected. The sequence of intruder types was alternated among colonies, and a 5-min interval separated introductions into any one colony. As the exact location of three field colonies could not be determined on day 27, only 12 colonies were tested at that time ($N=24$ introductions per treatment group).

Experiment 2

These experiments evaluated whether non-nestmates maintained on similar diets were less aggressive toward one another than non-nestmates maintained on dissimilar diets. Twenty-four monogyne field colonies were excavated. Queens were placed in vials with 10–30 workers to ensure queen safety and queen tending during transport to the laboratory. The remainder of each excavated colony (more than 10 000 workers) was re-established in the laboratory with original nest soil in large rearing trays ($64.0 \times 78.5 \times 9.5$ cm). Queens were reintroduced at this time. Colonies were provided with water tubes on the soil mound but were not supplied with food for 2 weeks. During this time, workers foraged on arthropods in the nest soil. Colonies were assigned randomly to one of two diet groups. After 2 weeks, separate foraging arenas were connected to each colony by a Tygon tube bridge. Twelve colonies received (ad libitum) honey-water and roaches (group I), while 12 received 5% sucrose solution and moth, *Anticarsia gemmatalis*, pupae (group II). Nest soil was watered throughout the experiment when necessary.

Aggression bioassays were conducted at 2 weeks (prior to presentation of diet) and at 5 months post-collection. In the pre-diet tests, colonies were tested against four within-group intruders (two each from

two colonies, $N=48$), four between-group intruders (two each from two colonies, $N=48$), and against two 'control' intruders from the resident colony being tested ($N=24$). Intruders were introduced onto the nest soil at least 10 cm from a nest opening. The sequence of intruder types was alternated among colonies on both days.

Based on brood production and the intensity of foraging, we concluded that only eight group I colonies and six group II colonies retained healthy, productive queens after 5 months in the laboratory. Therefore, we tested each of six colonies in either diet group against the other 11 for within- and between-group effects. To test recognition within diets, one intruder was introduced between each of the 30 (6 × 5) colony pairs in each of the two diet groups ($N=60$ introductions). Between-diet response was measured with two (reciprocal) introductions between each of 30 (6 × 5) paired colonies, one from each diet group ($N=60$ introductions). Tests were conducted on days 1 and 2 following the most recent feeding.

Experiment 3

This experiment determined whether the addition of an alien queen or non-nestmate worker for 28 days could detectably modify recognition labels or templates of recipient subcolonies. Minor and media workers and brood from monogyne field colonies ($N=14$) were established without queens in the laboratory for 10 weeks and then divided into four subcolonies of equal numbers of workers (range = 20–200 workers). One subcolony (Q+) was re-queened with a physogastric (i.e. highly fecund) field queen from a different site. This was accomplished by gently cooling the workers and introducing the field queen into the brood cell (orphaned *S. invicta* colonies readily accept replacement queens by this method). A control major worker from the field queen's colony of origin was added to another subcolony (W+) by repeated cooling and introduction. Two subcolonies, (AQ− and BQ−) were cooled, but received neither queen nor worker non-nestmates. Subcolonies were maintained on a honey-water and roach diet, and were covered with tight-sealing plastic tops during the course of the experiment. Pharate pupae (distinguishable by pigmentation) were removed from re-queened subcolonies starting at day 20, thereby ensuring that no subcolonies contained adult offspring of the alien queen. Aggression bioassays

Table II. Summary of nestmate recognition responses ($\bar{X} \pm$ SD) of (A) laboratory subcolonies to laboratory and field colony nestmates after 2 days and 27 days of laboratory rearing and (B) field colonies to laboratory and field colony nestmates on day 2 and day 27

	Intruder origin			
	Laboratory (*N*)	Field (*N*)	*T*	*P*
(A) Laboratory subcolony response				
Day 2	4·27±1·93 (30)	4·13±1·78 (30)	934·0	0·78
Day 27	3·54±2·34 (24)	5·88±1·62 (24)	728·5	0·002
(B) Field colony response				
Day 2	4·50±2·19 (30)	4·33±1·94 (30)	930·0	0·83
Day 27	6·13±2·42 (24)	3·29±2·44 (24)	749·5	<0·001

Significance levels (*P*) are based on the Wilcoxon rank sum, *T* (see Methods). The number of introductions are included in parentheses.

were conducted (as above) between days 25 and 28 to assess the effect of queen presence on worker–worker recognition. The following series of reciprocal introductions were performed within colonies: (1) control introductions (AQ− versus AQ− and BQ− versus BQ−), (2) queenless (AQ−) versus queenless (BQ−), (3) queenless (AQ− and BQ−) versus queen-added (Q+), and (4) queenless (AQ− and BQ−) versus worker-added (W+). Scores for each series of introductions represent the sum of four introductions per colony (i.e. reciprocal sets of two introductions). Thus, 56 nestmate introductions were conducted for each series of comparisons. Tests of AQ− versus BQ− and AQ− and BQ− versus Q+ were also conducted between colonies (*N*=2 non-nestmates introduced per colony). One series of introductions was conducted each day. In all tests, introductions into any one subcolony were spaced 5 min apart, and at least 90 min elapsed between the time a subcolony received intruders and the time it provided intruders.

RESULTS

Experiment 1

After 2 days in the laboratory, subcolonies exhibited no greater aggression toward field nestmates than they did toward laboratory subcolony nestmates (Table IIA). No intruders in either series

of tests were killed. However, after 27 days in the laboratory, subcolonies were significantly more aggressive toward field nestmates than toward laboratory subcolony nestmates (Table IIA). Although the magnitudes of aggression directed at laboratory-maintained nestmates on day 2 and day 27 were not significantly different (Wilcoxon test; *T*=736·0, *P*=0·19; Table IIA), laboratory colonies were more aggressive toward field nestmates on day 27 than on day 2 (Wilcoxon test; *T*=916·5, *P*<0·001; Table IIA). Twenty-one per cent (5 of 24) of the field nestmate intruders were killed.

Similar results were generated by the reciprocal presentation of laboratory-maintained workers to former nestmates in the field (Table IIB). On day 27, field colonies directed significantly more aggression toward laboratory-maintained workers than toward field nestmates. While no field nestmates were killed, 37·5% (9 of 24) of the laboratory-maintained nestmates were killed. Aggression directed at laboratory-maintained nestmates on day 27 was also greater than that directed at laboratory-maintained nestmates on day 2 (Wilcoxon test; *T*=808·5, *P*=0·01).

Experiment 2

After 2 weeks in the laboratory, workers introduced into their own colony (controls) elicited only 'investigative' behaviour (ranks 1–3, *N*=24). At this time, introductions between and within diet groups elicited similar levels of aggression (Table

Table III. Summary of responses ($\bar{X}\pm$SD) before diet was provided and after diet was provided of resident colonies to non-nestmate intruders reared on the same diet as residents or on a different diet

	Intruder diet			
	Same (N)	Different (N)	T	P
Before diet was provided	6·22±2·31 (45)	6·35±2·17 (45)	2146·0	0·42
After diet was provided	4·61±2·67 (54)	7·25±1·76 (54)	3513·0	<0·001

Significance levels (P) are based on the Wilcoxon rank sum, T (see Methods). The number of introductions are included in parentheses.

Table IV. Summary of nestmate and non-nestmate responses ($\bar{X}\pm$SD) elicited by reciprocal introductions between queenless subcolonies (Q− versus Q−) and queenless and queen-added subcolonies (Q− versus Q+)

	Subcolony pairing			
	Q− versus Q− (N)	Q− versus Q+ (N)	T	P
Nestmate	1·50±0·71 (56)	1·71±0·87 (56)	3384·0	0·20
Non-nestmate	3·14±2·12 (28)	2·96±1·90 (28)	803·5	0·93

Significance levels (P) are based on the Wilcoxon rank sum, T (see Methods). The number of introductions are included in parentheses.

III). (Note that introductions were excluded from analysis due to intruders taking refuge under or behind clumps of soil before all 20 interactions were recorded.) After 5 months of diet treatments, within-group introductions elicited significantly less aggression than between-group introductions (Table III). In addition, within-group aggression was reduced significantly with respect to pre-feeding levels (Wilcoxon test; $T=2674\cdot0$, $P=0\cdot002$; Table III). In contrast, aggression among ants reared on different diets was statistically unchanged (although somewhat increased) from pre-diet levels (Wilcoxon test; $T=2485\cdot5$, $P=0\cdot10$; Table III).

Experiment 3

All queens added to recipient subcolonies were accepted, and all produced viable brood during the tests. No major worker controls were found dead in any subcolony for up to 48 h post-introduction, and we assumed that all remained alive and integrated into their new colonies during the course of the experiment. Results of nestmate introductions are summarized in Table IV. Aggression scores for between-treatment introductions were not significantly different, with almost all introductions (162 of 168) between nestmate subcolonies eliciting only 'investigative' behaviour (ranks 1–3). No significant difference in recognition response was detected when reciprocal introductions between queenless and queen-added subcolonies were compared (Table IV), or when reciprocal introductions were compared between queenless subcolonies (mean=1·50, SD=0·71) and worker-added subcolonies (mean=1·46, SD=0·63; Wilcoxon test, $T=3175\cdot0$, $N_1=N_2=56$, $P=0\cdot96$). The magnitude of aggression elicited by queenless intruders introduced into queen-added subcolonies (mean=1·61, SD=0·99) was not significantly different from that elicited by queen-added intruders introduced to queenless nestmates (mean=1·79, SD=0·88; Wilcoxon test, $T=870\cdot0$, $N_1=N_2=28$, $P=0\cdot24$).

As with tests between nestmates, exposure of residents and intruders to an alien queen did not affect significantly the results of reciprocal introductions between non-nestmate subcolonies (Table IV). When only queen presence or absence in resident colonies was considered, workers from

queen-added and queenless subcolonies were equally aggressive toward non-nestmate intruders (Wilcoxon test; $T=429\cdot0$, $N_1=14$, $N_2=42$, $P=0\cdot57$). However, non-nestmates elicited more pronounced aggression than nestmates when introduced between queenless subcolonies (Wilcoxon test; $T=1517\cdot5$, $P=0\cdot002$; Table IV) and between queenless and queenright subcolonies (Wilcoxon test; $T=1493\cdot0$, $P=0\cdot004$; Table IV).

DISCUSSION

Workers in monogyne *S. invicta* field colonies vigorously attack intruders from neighbouring, conspecific colonies. Our study assumes that the magnitude of this aggression is positively correlated with the extent of perceived differences in recognition labels between two colonies. Partial justification for this assumption is provided by the fact that *S. invicta* workers are more aggressive toward members of other *Solenopsis* species than they are toward conspecifics (Obin 1987). Aggressiveness between conspecific colonies may also be affected by factors other than differences in recognition cues (Wallis 1962; Wilson 1971; Hölldobler 1976; Davies & Houston 1984; Jaffe & Puche 1984), and olfactory discrimination itself may vary with time of day (Hangartner et al. 1970). Recognizing the potential problems presented by colony, caste and contextual variation in aggression thresholds and fighting capability, we restricted our choice of 'intruders' to the reserve subcaste (Mirenda & Vinson 1981) and tested colonies at the same time of day. We also refrained from comparing aggression scores obtained in different experiments.

Environmentally Correlated Cues

Our laboratory and field bioassays indicate that colony level recognition in *S. invicta* is mediated in large part by environmentally correlated cues. In experiment 1, aggression between laboratory-reared and field-reared nestmates was increased significantly within 27 days of laboratory rearing. As in honey bees (Kalmus & Ribbands 1952), other ants (Wallis 1962; Jutsum et al. 1979), and as suggested for vertebrates (Galef 1981; Hepper 1986), group recognition cues used by *S. invicta*

workers can be derived (either directly or indirectly) from diet. In the present study, diet significantly modified worker labels in large, productive queenright colonies in original nest soil (experiment 2). Aggression between non-nestmates was reduced by maintaining colonies on the same diet, and aggression between colonies maintained on different diets was not significantly different from that observed when colonies were first collected.

A changing environment and the importance of environmentally correlated recognition cues in *S. invicta* suggests that worker labels are dynamic, and that updated recognition templates must be learned by workers throughout their lifetime (see also Wallis 1963; Vander Meer, in press). If labels can change more rapidly than templates, the possibility exists for false-negative recognition of nestmates as intruders. Such recognition errors may be evident in the high levels of aggression directed at *S. invicta* foragers returning to their colony after contacting novel food (M. Obin, unpublished data; see also Wallis 1963).

Diet-derived recognition cues are especially significant, because they are consistent with the hypothesis that 'appeasement' trophallaxis between non-nestmate workers promotes the formation and maintenance of polygyne (multiple-queen) *S. invicta* societies (Bhatkar 1979; Obin 1987). Controversy surrounds current evolutionary explanations of polygyny in the Formicidae (Hölldobler & Wilson 1977; Fletcher & Ross 1985), and the origin and explosive increase of polygyne fire ant colonies in the United States has received much recent attention (examples in Fletcher 1983; Ross et al. 1987b). Polygyny and nestmate recognition are intriguingly linked in fire ants, with recognition capability reduced relative to that observed in monogyne colonies (Mirenda & Vinson 1982; L. Morel, M. Obin & R. Vander Meer, unpublished data; other examples in Breed & Bennett 1987). During appeasement trophallaxis, intruding workers act as 'satellites' (Davies & Houstin 1984), exchanging food for access to a foreign territory or nest. The consequent sharing of diet-derived recognition cues among non-nestmates, could, under a model of 'habituated label acceptance' (Getz 1982), or 'cue similarity' (Gamboa et al. 1986a, b; Getz & Chapman 1987) promote the functional integration of non-nestmate worker forces, thereby leading to polygyny (Obin 1987). A similar mechanism may also facilitate colony desertion and intercolonial adoption

observed in the ant genus *Myrmecocystus* (Bartz & Hölldobler 1982) and between incipient *S. invicta* colonies (Tschinkel 1987).

Genotype-correlated Cues (Discriminators)

Although similar environmental cues can potentially eliminate recognition of non-nestmates in the leaf-cutting ant *Acromyrmex octospinosus* (Jutsum et al. 1979) and in the social wasp *Polistes fuscatus* (Gamboa et al. 1986a), aggression persists among non-nestmate *S. invicta* reared under similar laboratory conditions (Obin 1986; present study). This fact suggests that genotypically correlated cues or 'discriminators' (Hölldobler & Michener 1980) constitute some portion of worker recognition labels and templates. These discriminators could originate from queens or workers.

There were good reasons to suspect that fire ant queens transferred heritable, queen-specific odorants to workers. Queens are much larger than workers, they release several different queen pheromones within the colony (reviewed in Fletcher 1986; Glancey 1986), and the individual specificity of one of these queen pheromones had been suggested previously (Jouvenaz et al. 1974). If fire ant queen discriminators are transferred to workers and constitute some portion of the colony label, reciprocal introductions among recipient (queen-added) and non-recipient (queenless) colonies should elicit more pronounced aggression than introductions among non-recipient colonies. If labels alone were affected by the addition of a queen, we might expect an asymmetry in the direction of aggression directed at former nest-mates, with queenless colonies more aggressive toward intruders from queen-added groups than vice versa. Alternatively, if queen presence modified worker templates (or heightened worker motivation for nest defence), but queen discriminators were not transferred to workers, queen-added subcolonies might be more aggressive toward queenless intruders than vice versa. This rationale formed the basis of tests between queenless and worker-added subcolonies. In addition, comparison of queen-added and worker-added trials might indicate whether or not effects observed with queen-added groups were due specifically to the presence of queens.

However, addition of an alien queen or alien worker to small, queenless laboratory subcolonies did not significantly increase aggression between recipient and non-recipient subcolonies (experiment 3). These results argue against the effects of 'queen discriminators' (Carlin & Hölldobler 1983, 1986, 1987; Carlin et al. 1987) in experiment 1, and suggest that under the experimental protocol employed, adult *S. invicta* workers do not incorporate queen discriminators into the recognition template mediating colony defence (see also Mintzer 1982; Stuart 1985). Furthermore, the fact that the intensity of aggression directed toward non-nestmates by workers from queenless and queen-added colonies was not significantly different suggests that, within the detection limits of the bioassay, motivation underlying nest-defence aggression in small colonies of fire ants is independent of direct queen influence.

It should be noted that our data do not address the possibility that queen discriminators are learned during a critical period early in worker life (Brian 1986) or that conditioning by adult workers to compounds of newly introduced queens requires more than the 28 days that experiment 2 lasted. If either is found to be the case, it would suggest that queen discriminators and environmentally derived cues are incorporated into worker templates by different neural processing.

The importance of heritable recognition cues for close-kin recognition in social insects and thus for kin selection theory in general (review in Gadagkar 1985), has tended to obscure our appreciation of the variety of means by which recognition can be achieved. Fire ants, not unlike other social Hymenoptera studied (reviews in Gadagkar 1985; Breed & Bennett 1987), use both genetic and environmentally derived nestmate recognition cues. In the present study, the latter were sufficient for full recognition response. This result conforms to our emerging expectation that 'unifying cues' shared by all workers should be important in species with large colonies (e.g. *S. invicta*), and particularly in the context of colony-level activities such as nest defence (Breed & Bennett 1987; Hölldobler & Carlin 1987). Although Hölldobler & Michener (1980) originally suggested the queen as the logical source for such unifying cues in monogyne societies, it can be argued that environmental factors are equally reliable determinants of group and kin recognition cues in societies where group membership and kinship are highly correlated (Hepper 1986). This correlation is expected in monogyne *S. invicta* colonies, since queens are monoandrous (Ross & Fletcher 1985). The neglig-

ible contribution of queen discriminators to the fire ant colony label might also reflect the workers' obligate sterility (Hölldobler & Carlin 1987), with queens representing less of a chemical (olfactory) presence within the colony than queens of species exhibiting strong queen suppression of worker oviposition (e.g. *Camponotus*). Finally, limited introduction events and consequent genetic bottlenecks (Tschinkel & Nirenberg 1983; Ross et al. 1987a) may have reduced additive genetic variance for queen discriminator production in North American *S. invicta* populations. Worker discriminators could be similarly affected. A comparative study of nestmate recognition in imported and native (South American) *S. invicta* would address this possibility.

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