



Context-dependent nestmate discrimination and the effect of action thresholds on exogenous cue recognition in the Argentine ant

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The optimal acceptance threshold model predicts that kin/nestmate discrimination is context dependent and that, in a fluctuating environment, the action component of nestmate discrimination is plastic, rather than static. We examined changes in intraspecific aggression among colonies of Argentine ants, *Linepithema humile*, in various discrimination contexts, and found that aggression occurred at higher rates when either nestmates or familiar territory indicated nest proximity, but not when social context was absent, thereby providing additional support for the optimal acceptance threshold model. Context-dependent aggression in the Argentine ant appears to result from a shift in acceptance threshold in response to fitness costs associated with accepting nonkin. The change in the action component of Argentine ant nestmate discrimination was explained to some degree by the hypothesis that the presence of nestmates indicates nest proximity and denotes a fitness payoff for active defence (nest indicator hypothesis) and by the hypothesis that nestmates share the cost of nest defence in groups, but not singly (cost minimizer hypothesis). Isolated nest referents (familiar territory, conspecific brood, or single familiar nestmates), however, had no effect on aggression thresholds. We provide mixed support for the hypothesis that workers from genetically less diverse colonies attack workers from more diverse colonies. We found that, in the context of nest defence, genetically more diverse colonies initiated attacks on colonies with lower genetic diversity. Therefore, the role of asymmetrical aggression in reducing genetic diversity within introduced populations of *L. humile* remains unknown and other extrinsic factors such as nest status and/or colony size may affect the outcome of aggressive interactions in the field. Finally, our finding that colonies reared under uniform conditions showed diminished intraspecific aggression only when assayed in a social and/or ecological context underscores the importance of using appropriate aggression assays for testing patterns of intercolony aggression in *L. humile*, and that the use of different rearing regimes, source colonies and collection times may produce contradictory results.

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Intraspecific recognition in animals is manifested on several levels, including species, mate, social group, individual, or kin recognition. Kin recognition, as it relates to nepotism (preferential treatment of kin) and mate selection (avoidance of inbreeding and outbreeding) has evolved independently in many animal taxa (Sherman & Holmes 1985; Fletcher & Michener 1987; Hepper 1991). Several indirect (recognition based on nonphenotypic

cues) and direct (recognition based on phenotypic cues) mechanisms of kin recognition have been proposed (Blaustein 1983; Waldman et al. 1988). In one direct mechanism, phenotype matching, which is used by many animal taxa (Porter et al. 1983; Gamboa et al. 1986b; Sun & Müller-Schwarze 1997), individuals compare the phenotype of previously unencountered animals with an inner template (Lacy & Sherman 1983).

The phenotype matching process consists of three distinct components: (1) expression, (2) perception and (3) action (Sherman & Holmes 1985; Gamboa et al. 1986a; Waldman 1987; Reeve 1989). Expression can be any aspect of the phenotype that signifies membership reliably, as well as the mechanisms involved in cue production and

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acquisition. Perception involves the development of the recognition template, the processing of perceived cues, and the algorithm used to match cue with template. Finally, action is taken when cue and template match (Gamboa et al. 1986a). The action taken by the discriminating individual can be context dependent (Reeve 1989; Gamboa et al. 1991a), whereby the encountered conspecific might be tolerated in one recognition context and rejected in another.

In social insect societies where individuals frequently live in large and complex colonies, the constant exchange of information through an array of sophisticated social behaviours acts to synchronize interactions between individuals, thereby promoting efficient foraging, reproduction and defence. Eusocial insects maintain territorial boundaries aimed at preventing heterospecifics, as well as conspecifics, from invading and exploiting the colony's nest. Individuals inhabiting a nest are identified through a process of nestmate recognition, whereby nestmates display differential treatment towards conspecifics according to their nest of origin (Gamboa et al. 1986b). In most groups of social insects, nestmates are usually kin and nestmate and kin recognition are generally assumed to be equivalent and involve similar modes of discrimination (Gamboa et al. 1991a).

Recognition cues are dynamic and may change throughout the life of the colony (Vander Meer et al. 1989; Provost et al. 1993) and vary seasonally (Ichinose 1991). In addition, nestmate recognition can vary with colony size (Stuart 1991), stage (Balas & Adams 1996) and queen number (Keller & Passera 1989; Starks et al. 1998a). Such variation in genetic and environmental parameters requires that colony members continually obtain and process information about their changing ecological and social environment and act accordingly. This requires action component plasticity to minimize the chance of recognition errors. Reeve (1989) developed the optimal acceptance threshold model to describe kin discrimination plasticity, whereby the acceptance threshold varies according to context, to balance the fitness costs of accepting nonkin and rejecting kin. Therefore, as the cost of accepting nonkin increases, the acceptance threshold is lowered.

For many animals, the proximate mechanisms by which individuals recognize kin are well understood, but the context dependence of such mechanisms is rarely tested. Herein, we use the Argentine ant, *Linepithema humile*, as a model organism to study the plasticity of recognition systems. The Argentine ant is an invasive species native to South America. In its native range, *L. humile* is multi-colonial, maintaining distinct territorial boundaries, with aggression between colonies occurring across relatively small spatial scales (Suarez et al. 1999; Tsutsui et al. 2000). Within introduced populations, however, *L. humile* is unicolonial, producing large multiple-queen colonies that lack clear boundaries due to a general absence of intraspecific aggression (Newell & Barber 1913; Suarez et al. 1999; Tsutsui et al. 2000; Giraud et al. 2002). Argentine ants respond to both genetically based recognition cues (Tsutsui et al. 2000; Suarez et al. 2002) and cues derived from the environment (Chen & Nonacs 2000; Liang &

Silverman 2000). The relative importance of *L. humile* heritable and exogenous nestmate recognition cues was studied under constant laboratory conditions, where changes in aggression patterns between individuals from different colonies were assessed. Chen & Nonacs (2000) recorded a complete loss of aggression in colonies reared for 2 months under uniform laboratory conditions, which suggests a primary role for environmentally derived cues. In contrast, Suarez et al. (2002) and Holway et al. (1998) detected little change in the pattern of intraspecific aggression over time and concluded that the role of exogenous cues was minor, relative to inherited cues. Possible explanations for the disparate study conclusions include distinct laboratory rearing regimes (e.g. dietary and nesting conditions) as well as the length of time colonies were maintained in the laboratory (3 weeks to >1 year). Also, different source colonies and collection times as well as distinct aggression tests to estimate changes in patterns of nestmate discrimination may have produced different results.

While differences in colony source and phenology may account for the relative contributions of heritable and exogenous nestmate recognition cues, we suspect that the social context in which aggression behaviour was examined strongly affected the action component of discrimination as per Reeve's (1989) model, with conspecific discrimination being sensitive to experimental conditions. Therefore, although heritable cues may dominate nestmate recognition in Argentine ants, we hypothesize that the contribution of exogenous cues becomes evident when the action threshold is lowered during critical colony activities, such as nest defence. The choice of aggression assay can significantly affect estimates of intercolony aggression, with assays placing ants in the context of nest defence most likely to reveal aggression (Roulston et al. 2003). Herein, we attempt to reconcile differences between earlier conclusions regarding the relative contribution of heritable and environmental nestmate recognition cues in *L. humile* by examining intraspecific aggression between identical source colonies reared under uniform laboratory conditions and tested in different social contexts. We compared levels of aggression between non-nestmates in three different aggression assays: (1) intruder introductions into an established resident territory, (2) symmetrical group interactions in a neutral arena, and (3) dyadic interactions between workers within a neutral arena. In intruder introductions, an intruder worker was introduced into an established nest containing a resident colony. In this assay, the nestmate recognition context was apparent, with resident workers discriminating within a familiar territory and in close proximity to the nest. Non-nestmates represent an usurpation pressure; consequently, nestmates may be more aggressive towards non-nestmates when the nest is present. Also, high worker density generally indicates that the nest is nearby. In the symmetrical group interaction assay, worker cohorts interacted in a neutral arena apart from their nests. Although without the nest, workers may be less inclined to attack non-nestmates when there are no resources to protect, we predicted that workers would be aggressive towards non-nestmates because increasing

worker density indicates nest proximity. Furthermore, we predicted that the presence of familiar nestmates would facilitate aggression towards non-nestmates when the cost of defence was shared with other nestmates. In dyadic interactions, the context of colony defence was absent because workers were isolated from both nestmates and a familiar territory and the threat of resource usurpation was low. Therefore, we predicted that the chance of detecting aggression, and consequently of detecting declines in aggression after colony rearing under constant environmental conditions, would be less than in the contexts allowing group interactions.

In addition, we tested two hypotheses proposed by Starks *et al.* (1998b): (1) the nest indicator hypothesis, which predicts that the presence of nestmates indicates nest proximity and denotes a fitness payoff for active defence, and (2) the cost minimizer hypothesis, which predicts that nestmates are willing to share the cost of nest defence in groups, but not singly.

We also reexamine the hypothesis of Tsutsui *et al.* (2003) that individuals from genetically less diverse colonies attack individuals from more diverse colonies. Using neutral arena behavioural tests (dyadic interactions), Tsutsui *et al.* (2003) demonstrated such polarized aggression and proposed it as a mechanism leading to supercolony formation in *L. humile*. Our preliminary observations indicated that, in intruder introductions, the residents are protective of their territory and often initiate attacks on the intruders. The importance of a familiar territory for defence has been explicitly demonstrated in numerous studies (e.g. Hölldobler 1976; Davies 1978; Hölldobler & Lumsden 1980; Gordon 1989). Therefore, we hypothesized that nest status of individual ants in intruder introductions (residents versus intruders), rather than the level of genetic disparity between individuals, is of primary importance in determining the identity of the aggressor. To test this hypothesis, we compared colonies with different levels of genetic diversity (microsatellite alleles) and performed intruder introduction tests between colonies of low and high diversity.

METHODS

Collection and Maintenance of Laboratory Colonies

We used six colonies of Argentine ants collected in North Carolina, U.S.A.: Chapel Hill (CHH), Emerald Isle (EMI), Greenville (GNC), Jacksonville (JAC), Research Triangle Park (RTP) and Winston-Salem (FOR) and one colony from California: Pleasanton (PLS). For each location, we established a single colony consisting of 15 000–20 000 workers, a few hundred queens, and numerous brood. Colonies were maintained in soil-free, Fluon-coated trays containing nests composed of plastic dishes filled with moist grooved plaster. Colonies were provisioned with 25% sucrose solution and an artificial diet (Bhatkar & Whitcomb 1970) ad libitum and hard-boiled egg once a week. All colonies were maintained at $24 \pm 1^\circ\text{C}$ and $50 \pm 10\%$ RH on a 12:12 h light:dark cycle.

Aggression Tests

We assessed the initial level of aggression between 12 colony pairs (GNC-CHH, CHH-FOR, EMI-CHH, JAC-CHH, CHH-RTP, EMI-RTP, PLS-CHH, PLS-FOR, PLS-GNC, PLS-EMI, PLS-RTP, PLS-JAC) using three different aggression bioassays: (1) intruder introductions into an established resident territory, (2) group worker interactions in a neutral arena, and (3) worker dyad interactions within a neutral arena. Detailed descriptions of each assay are presented below. We then compared the results of these bioassays, each designed to measure context-dependent changes in aggression. For each assay, the observer who recorded worker aggression level did not know the identity of the interacting colonies and was unfamiliar with the hypothesis being tested. Individual ants were not used in more than one trial and estimates of initial aggression levels were determined within 1 week of collection. The same colony pairs were evaluated 4 months later for changes in aggression.

Assay 1: intruder introduction to resident territory

Individual intruder workers were collected on a toothpick and introduced into rearing trays ($52 \times 38\text{ cm}$) containing a resident colony ($\sim 10\,000$ workers). Aggression was scored using the 0–4 scale of Suarez *et al.* (1999). To avoid a possible confounding effect of increasing the probability of detecting aggression with more encounters between the intruder and the residents, we recorded the aggression score for the first encounter. The intruder was discarded after each trial, and subsequent trials were conducted when the residents were no longer visibly agitated (5–10 min). Ten replicates per colony pair were performed; five replicates with colony 1 as the resident and five replicates with colony 1 as the intruder.

Assay 2: symmetrical group interactions in a neutral arena

We followed the protocol of Chen & Nonacs (2000). We transferred 20 randomly selected workers to a plastic, Fluon-coated container (10 cm diameter, 5 cm high). Similarly, we placed 20 workers in a plastic Fluon-coated arena ($30 \times 17.5 \times 8.25\text{ cm}$ high). Both groups of ants were allowed to calm for 5 min, after which the two groups of workers were combined by gently emptying ants from the smaller tray into the arena. We performed 10 replicates per pairing. The number of ants involved in fighting (aggression level 3 or above) was recorded immediately (within 2–3 s) after ants from the two colonies were mixed. This amount of time was sufficient for the aggressive phenotypes to find each other and become locked in fights (usually pairs or triplets). The nonaggressive phenotypes did not participate in fights.

Assay 3: dyadic interactions

We collected two workers, selected at random from each of two stock colonies, on a toothpick and placed them sequentially into a glass vial (2-dram, 3.7 ml). Vials were Fluon-coated within 1 cm of the bottom, thereby restricting ants to a small area to maximize the chance of the ants

finding each other. Ant interactions were scored on a 0–4 scale (Suarez et al. 1999) and the aggression score for the first encounter was recorded.

Response of Same Individual in Different Social Contexts

In contrast to dyadic interactions, assays placing ants in the context of nest defence were most likely to reveal aggression (Roulston et al. 2003). To examine further the context dependency of aggression, we measured the aggressiveness of one individual by placing it in two different contexts: as a member of a dyadic pair and as an intruder. Using two colonies, Chapel Hill (CHH) and Pleasanton (PLS), which in earlier tests displayed aggression, we first assessed the level of aggression in dyadic interactions (60 replications). To distinguish workers from the two colonies during trials, we marked PLS workers by feeding them a small amount of dilute sugar water, which does not affect ant discriminatory capacity (Tsutsui et al. 2003). In instances where the two ants did not display aggression within 5 min, we removed the marked workers from the vial and introduced them into a rearing tray containing the opposing colony. We scored behavioural interactions between the intruder and the resident ants again as described in assay 1. To examine the change in behaviour in ants that did not show aggression in dyadic tests, we noted whether the intruder or the resident initiated aggression.

Effects of Familiar Territory, Familiar Nestmates and Brood on Aggressive Interactions

Starks et al. (1998b) reported that aggression towards non-nestmates in *Polistes dominulus* increases in the presence of either familiar nestmates (cost minimizer hypothesis) or a familiar nest fragment (nest indicator hypothesis). We measured aggression between two *L. humile* colonies, Cary (CAR) and Winston-Salem (FOR) in dyadic interactions (assay 1) and intruder introductions (assay 3) and also modified these assays to evaluate the relative importance of nestmates or brood. We determined the effect of nest and/or familiar territory by measuring aggression towards intruders by workers that were or were not isolated from their nest for 1 week. Approximately 2000 workers from a laboratory colony were transferred to a clean tray without queens or brood and were provisioned with 25% sucrose solution and a water vial stoppered with cotton, to prevent ants from using the vial as a nest. To test whether modifying the social context increases aggression between worker dyads, we included brood or an additional nestmate (triad) or a colony-specific odour. We transferred brood (two late-instar larvae and two pupae) into the vial with a brush, then added a nestmate worker. After 1 min, we added a non-nestmate worker and noted the behavioral interaction between the workers. We also recorded the number of workers that carried brood at any time during the trial. To precondition

the vials with colony-specific odour, we first coated the inner surface of each vial with Fluon, then placed the vials in stock colonies for 9 days. In the Argentine ant, marking of home ranges and/or territories has not been reported. However, home range and territory marking has been described in several other ants (Cammaerts et al. 1977; Hölldobler & Wilson 1977; Jaffe et al. 1979). We observed Argentine ants visiting objects that had not been previously exposed to the colony, possibly depositing chemicals that may aid in territory recognition. We measured aggression in tests with conditioned vials and triads as described in assay 3. We performed 50 replicates for each assay.

Territory Defence and Its Effect on the Polarity of Aggression

We performed intruder introduction tests (assay 1) between colonies having different numbers of microsatellite alleles to test whether nest status of individual ants (residents versus intruders), rather than the direction of allelic diversity asymmetry, is of primary importance in determining the identity of the aggressor. For microsatellite analysis, we extracted genomic DNA from 15 workers from each site using the DNeasy Tissue Kit (Qiagen, Valencia, California) and scored allelic diversity using seven polymorphic microsatellite loci: *Lhum-11*, *Lhum-13*, *Lhum-19*, *Lhum-28*, *Lhum-35*, *Lhum-39* (Krieger & Keller 1999) and *Lihu-T1* (Tsutsui et al. 2000). PCR reactions were multiplexed and amplification products were separated on 6.5% KB^{Plus} polyacrylamide sequencing gels using a 4000L Li-Cor DNA sequencer. Microsatellite alleles were scored using RFLPScan software (Scanalytics, Billerica, Massachusetts, U.S.A.). Two colonies, PLS and CHH with 17 and 16 alleles, respectively, were paired up against the remaining five colonies having 21 to 29 alleles. An observer, blind to the identity of the colonies and the hypothesis being tested, recorded the frequency of initiation of aggression by residents, intruders, or both. There were 20 replicates per colony pair, 10 replicates with the lower-diversity colony acting as the resident and 10 with the higher-diversity colony acting as the resident. The frequency of trials in which the intruder and the resident attacked simultaneously was relatively low (8 out of 200). Therefore, such trials were disregarded and replaced with a new replicate.

Statistical Analyses

We determined the effect of exogenous cues on nestmate recognition by measuring the change in aggression within each of the three social contexts using the PROC UNIVARIATE procedure (signed-ranks test) in SAS 8.1 (SAS 2002). This procedure was also used to measure changes in aggression in assays that examined the relative importance of additional nest referents on intercolony aggression. In all analyses, absolute rather than relative aggression loss values were used. The polarity of aggression between residents and intruders in dyadic interactions was tested using Wald's chi-square test.

RESULTS

Initial and 4-month intercolony aggression levels are presented in Table 1 and Fig. 1. All colony pairings revealed high initial aggression only with assays that permitted group interactions (symmetrical group interactions and intruder introductions). By contrast, dyadic interactions revealed only low and noninjurious aggression. Dyadic interactions and intruder introductions used the same scoring scale (0–4), therefore permitting a direct comparison of aggression scores. Aggression scores during intruder introductions ($\bar{X} \pm SE = 3.2 \pm 0.1$) were significantly higher than those during dyadic interactions (1.2 ± 0.1 ; t test: $t_{59} = 7.8$, $P < 0.0001$), demonstrating differences between assays and the context dependency of *L. humile* aggression. When the same individual worker was placed in different social contexts (dyadic interactions versus intruder introductions), 37 of 60 dyadic interactions revealed no aggression (score of 0) and the average aggression score for all replications was 0.77 ± 0.18 . When workers that did not show aggression in dyadic encounters were introduced into an opposing colony, high aggression ensued between the intruders and the residents (average score = 3.7 ± 0.1 ; t test: $t_{36} = -47.4$, $P < 0.0001$), demonstrating that low aggression in dyadic interactions was due to the lack of context and not chance selection of nonaggressive phenotypes.

The effect of additional nestmates (triads), familiar territory (nest), or brood on aggressive interactions are presented in Fig. 2. The standard intruder introduction assay (with nest) produced an average aggression score of 2.9 ± 0.2 and only three of 50 trials resulted in no aggression (score of 0). In contrast, dyadic interactions (without brood) revealed significantly lower aggression (0.5 ± 0.2) and 39 of 50 trials resulted in no aggression (Wilcoxon signed-ranks test: $t_{49} = 39.2$, $P < 0.0001$). Aggression was not diminished by isolating workers from their nest and a familiar territory ($t_{49} = 14.2$, $P = 0.546$). In dyadic interactions, the addition of brood reduced aggression from 0.5 ± 0.2 to 0.1 ± 0.05 ($t_{49} = 79.4$, $P = 0.004$). We observed that workers immediately picked up their own brood and carried the brood around within the vial, usually for the duration of the test. In 20 of 50 trials, both workers carried brood, in 22 trials, one of the workers carried brood, and in eight trials, none of the workers carried brood. Dyadic interactions in preconditioned vials were no more aggressive than interactions in clean vials ($t_{49} = -36.5$, $P = 0.486$). Similarly, triads were no more aggressive than dyads ($t_{49} = 10.5$, $P = 0.702$).

Table 1. Initial aggression levels and subsequent changes over a 4-month period in three social contexts

Aggression bioassay	Aggression levels*		Aggression loss		
	Initial	End	Absolute	Relative (%)	P†
Symmetrical group interactions	76.2 ± 3.6	53.3 ± 4.0	-22.9	-30.1	0.001
Intruder introductions	3.2 ± 0.1	2.1 ± 0.2	-1.1	-34.9	0.001
Dyadic interactions	1.1 ± 0.1	1.4 ± 0.1	+0.2	+13.7	0.23

*Values reported are means \pm SE.

†Absolute aggression loss values were used in statistical analysis (signed-ranks test).

In intruder introductions between colonies having disparate levels of genetic diversity, aggression was polarized relative to both allelic diversity and direction of worker introduction (Table 2; Wald's $\chi^2_1 = 30.2$, $P < 0.0001$). In colony pairings involving CHH workers, aggression was polarized with respect to nest status, but not genetic diversity. Intruding CHH workers were aggressed upon by the higher-diversity resident colonies in 96% of trials (48/50) (Wald's $\chi^2_1 = 35.9$, $P < 0.0001$). When the roles were reversed, resident CHH workers initiated aggression more often (Wald's $\chi^2_1 = 27.3$, $P < 0.0001$), attacking the intruders in 84% of trials (42/50). In colony pairings involving PLS workers, aggression was polarized with respect to genetic diversity, but not nest status. Intruder PLS workers initiated aggression more often than the residents in 74% of trials (37/50; Wald's $\chi^2_1 = 24.0$, $P < 0.0001$), whereas resident PLS workers initiated aggression more often than the intruders in 82% of the trials (41/50; Wald's $\chi^2_1 = 25.5$, $P < 0.0001$).

We detected significant changes in Argentine ant aggression following prolonged laboratory rearing for symmetrical group interaction and intruder introduction assays, but not for dyadic pairings (Table 1, Fig. 1). Symmetrical group interaction and intruder introduction assays revealed a loss in aggression in 10 (83%) and 11 (92%) colony pairs, respectively, whereas dyadic interactions revealed aggression declines in three (25%) colony pairings, increases in six (50%) pairings, and no change in three (25%) pairings.

DISCUSSION

We demonstrate that aggressive interactions between Argentine ant workers are affected by social and/or ecological contexts and that responses to externally derived recognition cues are context dependent. We provide additional support for Reeve's (1989) optimal acceptance threshold model, whereby the action component of nestmate discrimination in *L. humile* is flexible rather than static, so that as the fitness cost of accepting non-nestmates decreases, the aggression threshold increases, and the probability of rejecting non-nestmates decreases.

We provided social and ecological contexts that varied from minimal (or possibly even absent) to one approximating field conditions. As a consequence, aggression between non-nestmates was high in group interactions, where the fitness cost of accepting nonkin was high, and

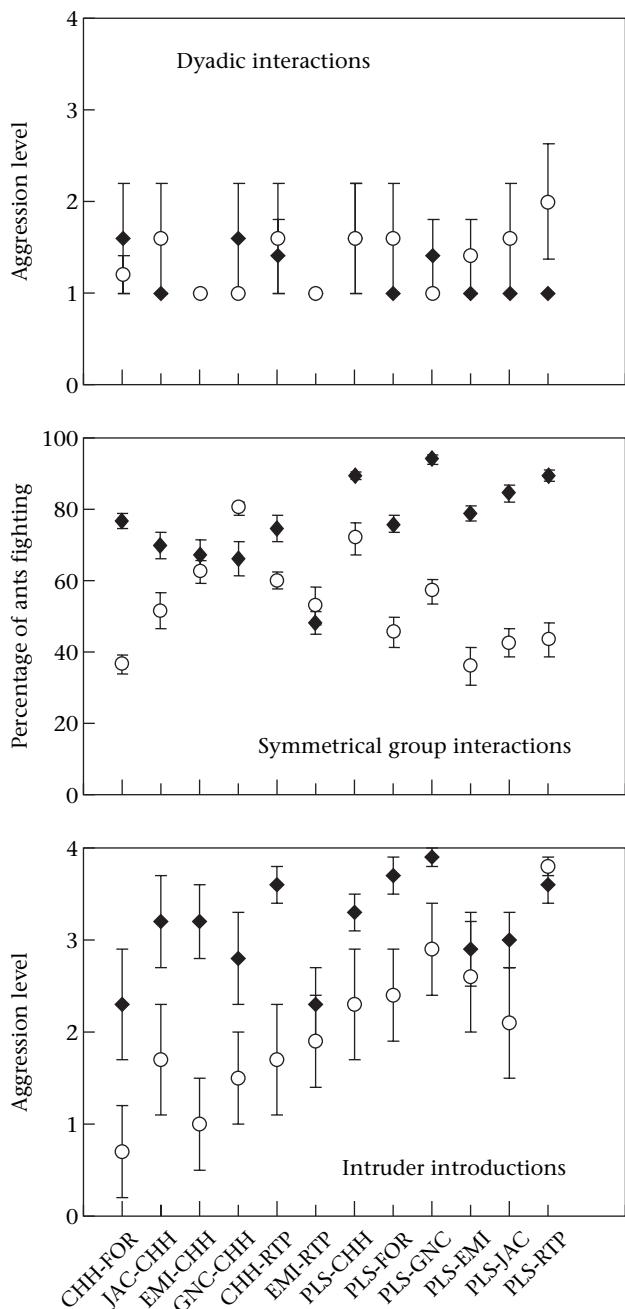


Figure 1. Mean \pm SE aggression levels for colony pairings at the beginning (●) and at the end (○) of 4 months of laboratory rearing. See Table 2 for source colony abbreviations.

low (8% of encounters) in dyadic pairings, where the cost of accepting nonkin was low.

Starks et al. (1998b) hypothesis in *Polistes dominulus* (Hymenoptera: Vespidae) found strong support for the nest indicator, but not for the cost minimizer hypothesis. Context-dependent aggression and the importance of a familiar territory for social organization and defence is evident in various social insects (Hölldobler 1976; Hölldobler & Lumsden 1980; Pfennig & Reeve 1980; Gordon 1989). Jaffe (1986) suggested that territory and nestmate

recognition cues are identical and Vander Meer et al. (1989) demonstrated a constant exchange of exogenous and endogenous cues between colony members and the environment. Also, aggression towards non-nestmates increases with increasing proximity to the nest (Gamboa et al. 1991b; Venkataraman & Gadagkar 1992; Starks et al. 1998b).

In *L. humile* dyadic encounters, the absence of familiar nestmates may signal that the nest is relatively distant and individuals thereby perceive no threat of colony resource usurpation. Furthermore, the cost of nest defence is not shared with nestmates; consequently, individuals may be reluctant to engage in aggressive behaviour. Also, we frequently observed workers attempting to escape the vial, indicating that their priorities may switch from guarding the colony to ensuring their own safety within an unfamiliar environment. Results from our symmetrical group interactions, where workers from both colonies fought despite the absence of familiar nest cues, support the cost minimizer hypothesis: individuals in the presence of nestmates were more aggressive when the cost of nest defence could be shared. Evidence of high aggression of resident workers towards intruders supports both nest indicator and cost minimizer hypotheses.

Context dependency of aggression in *L. humile* was apparent when the same individual worker was placed in different social contexts (dyadic interactions versus intruder introductions). Workers that did not show aggression in dyadic interactions displayed high aggression in intruder introductions. The general lack of aggression across dyadic encounters may result either from selection of one or two nonaggressive workers or from inhibition of aggression due to the lack of context. When two randomly selected ants are nonaggressive, the prospect of detecting aggression is indeed very low. When one of the ants is nonaggressive, submissive behaviour by one worker may not provoke aggression in the other because the behaviour of each participant in a dyadic interaction is a function of both the template-cue match and the behaviour of the participants (Gamboa et al. 1991a). We show that lack of aggression in *L. humile* dyads is not because of selection of one or two nonaggressive phenotypes, but rather is possibly due to other factors such as the unwillingness of workers to incur the full cost of aggression in the absence of nestmates when they are away from the nest.

Although aggression towards non-nestmate *P. dominulus* increases in the presence of either a familiar nest fragment or familiar nestmates (Starks et al. 1998b), we recorded no independent effect of familiar territory, presence of brood, or presence of one additional nestmate on Argentine ant aggression. Social wasps construct and maintain elaborate paper nests throughout an entire season and use cues originating from both the brood and the nest in nestmate recognition (Ross & Gamboa 1981; Shellman 1982; Gamboa et al. 1986a; Singer & Espelie 1992); therefore, factors associated with the nest may be important territorial cues. In contrast, Argentine ants create impermanent nests, moving frequently in response to changes in the physical environmental (Newell & Barber 1913) and the distribution of food resources (Holway & Case 2000; Silverman & Nsimba 2000). Therefore, the importance of nest material in *L. humile* territorial defence may be negligible.

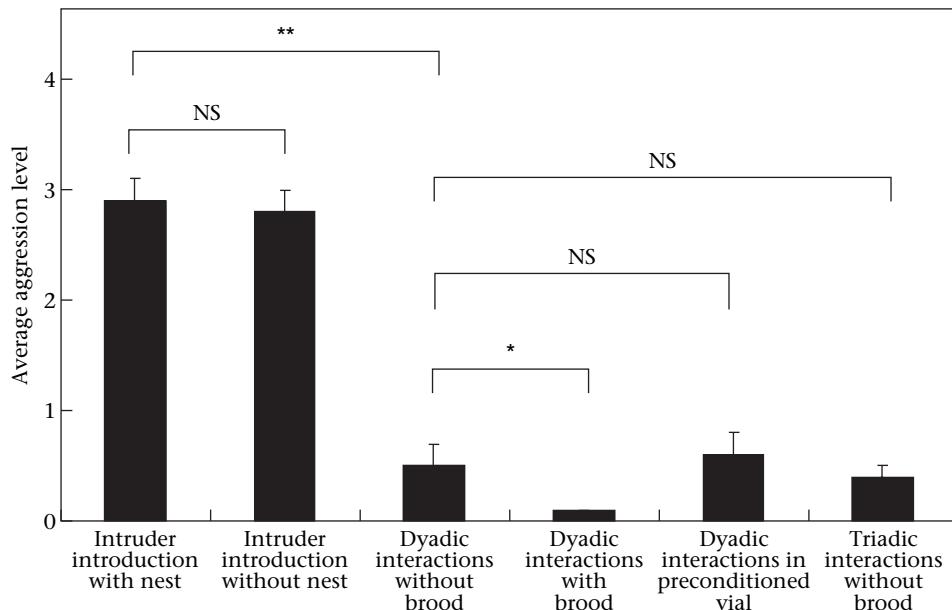


Figure 2. Mean \pm SE aggression levels between non-nestmates in different contexts. $N = 50$. * $P < 0.01$; ** $P < 0.0001$.

Contrary to our predictions, the addition of conspecific brood reduced aggression between non-nestmates. Brood-carrying behaviour was largely responsible for the observed reduction in aggression and the presence of brood may have caused one or both workers to switch priorities from fighting rivals to finding a secure place to deposit brood. Also, because workers used their mandibles to grasp brood, this may have effectively prevented the workers from engaging in fights.

We predicted that colony-specific odours would signal the proximity of the nest, providing a fitness payoff for protection of relatives, but no increase in aggression in preconditioned vials was observed. Although species-specific home-range marking occurs in ants (Mayade et al. 1993; Jaffe et al. 1979), including the Argentine ant (Aron et al. 1990), colony-specific territory marking observed in various ants (reviewed in Hölldobler & Wilson 1990) has not been reported in the Argentine ant. We also

considered that although the appropriate colony-specific cues may have been present, Argentine ant worker aggression was low due to the reluctance to engage in territory protection when familiar nestmates were absent.

Although support for the cost minimizer hypothesis (Starks et al. 1998b) comes from increased aggression in triadic versus dyadic interactions between *P. dominulus* workers, we observed no elevated aggression in Argentine ant triads. However, elevated *L. humile* aggression in large symmetrical group interactions suggests that there may be a group size threshold required to provoke aggression, possibly related to nest defence.

We provide evidence for factors other than disparities in genetic diversity driving asymmetrical aggression in Argentine ants. Tsutsui et al. (2003) suggested that selection against rare alleles at recognition loci may promote uniclonality in Argentine ants. Using worker dyads, Tsutsui et al. (2003) reported asymmetrical aggression,

Table 2. The frequency of aggression between intruders and residents in colony introductions

Intruder	Resident	Allelic ratio	Attacks by intruders	Attacks by residents	Intruder	Resident	Allelic ratio	Attacks by intruders	Attacks by residents
CHH (16)	EMI (26)	0.62	0	10	EMI (26)	CHH (16)	1.63	2	8
CHH (16)	FOR (21)	0.76	1	9	FOR (21)	CHH (16)	1.31	0	10
CHH (16)	GNC (25)	0.64	0	10	GNC (25)	CHH (16)	1.56	2	8
CHH (16)	JAC (27)	0.59	0	10	JAC (27)	CHH (16)	1.69	1	9
CHH (16)	RTP (29)	0.55	1	9	RTP (29)	CHH (16)	1.81	1	9
Total			2	48				6	44
PLS (17)	EMI (26)	0.65	7	3	EMI (26)	PLS (17)	1.53	3	7
PLS (17)	FOR (21)	0.81	7	3	FOR (21)	PLS (17)	1.24	0	10
PLS (17)	GNC (25)	0.68	7	3	GNC (25)	PLS (17)	1.47	2	8
PLS (17)	JAC (27)	0.63	8	2	JAC (27)	PLS (17)	1.59	3	7
PLS (17)	RTP (29)	0.59	8	2	RTP (29)	PLS (17)	1.71	1	9
Total			37	13				9	41

CHH: Chapel hill; EMI: Emerald Isle; FOR: Winston-Salem; GNC: Greenville; JAC: Jacksonville; RTP: Research Triangle Park; PLS: Pleasanton. Numbers in parentheses are the total number of alleles across seven microsatellite loci.

whereby workers from genetically less diverse colonies attacked workers from more diverse colonies, and attackers were six times more likely to survive agonistic encounters than recipients of aggression. However, in the context of nest defence, we found that genetically more diverse colonies initiated attacks on colonies with lower genetic diversity. Thus, nest status is an important additional factor that may drive intraspecific aggression in *L. humile*. Surprisingly, however, genetically more diverse colonies initiated attacks only in pairings involving CHH ants, and not in those involving PLS ants. While attackers that have low genetic diversity are more likely to win in dyadic fights (Tsutsui et al. 2003), lone intruders introduced into resident colonies are always killed, irrespective of the level of genetic diversity, simply because they are outnumbered by the resident workers. Under natural conditions, the sizes of competing Argentine ant colonies are likely to be unequal and fights are likely to involve groups of workers rather than dyads. In such situations, the odds of winning may be primarily influenced by asymmetry in colony size rather than asymmetry in colony allelic diversity. While further evidence is required to determine the relative importance of territorial status versus genetic diversity in intraspecific competition, we suggest that more genetically diverse Argentine ant colonies may not be displaced by their less diverse neighbours.

We also demonstrated that cues derived from the Argentine ants' external environment contribute to the overall recognition profile in this species. However, assays using intercolony dyads may underestimate the importance of exogenous cues in nestmate recognition if acceptance thresholds are not exceeded both before and after a period of colony maintenance under constant environmental conditions. Thus, the differing conclusions of Chen & Nonacs (2000) and Suarez et al. (2002) regarding the contribution of environmentally derived cues to nestmate recognition can be reconciled, at least in part, by considering the context in which intercolony aggression was monitored in each case. Our data from colonies assayed for aggression in different social contexts support the conclusions of both Chen & Nonacs (2000) and Suarez et al. (2002); that is, there was a temporal reduction in aggression during group interactions (Chen & Nonacs 2000), but not during dyadic pairings (Suarez et al. 2002). It is important to acknowledge, however, that the results of Chen & Nonacs (2000) and Suarez et al. (2002) cannot be fully reconciled based on differences in aggression testing methodology alone. Other factors such as distinct laboratory rearing regimes, different source colonies and collection times may have also contributed to contradictory conclusions regarding the role of genetic versus environmental inputs for nestmate recognition in *L. humile*.

Our results demonstrate the importance of using appropriate aggression assays for evaluating changes in inter-colony aggression patterns. Acceptance thresholds are context specific, which entails that nestmate discrimination is sensitive to experimental conditions. We show that aggression is a function of the fitness consequences of accepting or rejecting conspecifics. Therefore, we suggest that investigators be alert to the context dependency of aggressive behaviour when investigating conspecific

discrimination in neutral experimental arenas. We recommend the use of assays that involve group fights and present realistic aggression contexts.

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