

Research article

Contact between supercolonies elevates aggression in Argentine ants

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Abstract. Complex recognition systems underlie the social organization of many organisms. In social insects the acceptance of other individuals as nestmates can involve a variety of different cues, but the relative importance of these cues can change in relation to the fitness costs of accepting or rejecting other individuals. In this study we investigate the mechanisms that underlie recognition behaviour in Argentine ants (*Linepithema humile*). Introduced populations of Argentine ants are characterized by a social structure known as unicoloniality where intraspecific aggression is absent over large distances resulting in the formation of expansive supercolonies. Recent research has identified sites where multiple, mutually aggressive supercolonies co-occur allowing an examination of Argentine ant behaviour at territorial boundaries. We found that workers from different supercolonies always interact aggressively with one another, but that neighbours from different colonies (i.e., workers from nests located in the immediate vicinity of territory borders) consistently exhibited higher levels of aggression compared to those displayed by non-neighbours from different colonies (i.e., workers from nests located far enough away from a territory border so that interactions are unlikely). This difference in the level of aggression displayed between neighbours and between non-neighbours from different supercolonies cannot be explained by differences in relatedness or genetic similarity. Instead our findings suggest that direct contact between mutually antagonistic colonies is sufficient to elevate aggression. A laboratory experiment in which we manipulated the extent to which colonies with no prior history of contact could interact with one another, revealed that aggression increased after colonies were permitted to interact, but dropped after connections between colonies were severed. Moreover, the mere presence of an aggressive supercolony was sufficient to

elicit elevated aggression. Overall these patterns are opposite to the “dear enemy” phenomenon and could be the result of the intense territorial aggression exhibited by established supercolonies of this species.

Keywords. Nestmate recognition, dear enemy, territory border, context-dependent, genetic relatedness.

Introduction

The organization of individual organisms into cohesive social groups requires, above all, the ability to distinguish group members from non-members. In many cases, recognition systems are based on “phenotype matching” (Lacey and Sherman, 1983), a process by which one individual assesses how well the phenotypic label (or “cue”) of another individual matches the suite of cues (or “template”) that characterize other members of the social group (reviewed in: Crozier and Dix, 1979; Breed, 1983; Breed and Bennett, 1987; Ratnieks, 1991; Sherman et al., 1997; Mateo, 2004; Tsutsui, 2004). Templates are typically a neural or cognitive representation of the phenotypes that distinguish group members, and they often develop through processes of learning or imprinting. Although phenotype matching forms the basis of many recognition systems, the behaviour displayed towards individuals that are not group members can be quite plastic and shift in response to changing social environments. For example, in a theoretical analysis of recognition behaviour in a variety of different social contexts, Reeve (1989) predicted that aggression should be displayed more frequently toward non-kin, and that such rejection should increase when encounters between “non-self” individuals are more frequent. Accordingly, a number of studies in

widely divergent taxa have described how different recognition behaviours are displayed in different contexts (e.g. *Pocillopora* coral, Hidaka, 1985; red-legged frog larvae, Blaustein et al., 1993; spadefoot toad larvae, Pfennig et al., 1993; encrusting marine bryozoans, Shapiro, 1996; *Stylophora* coral, Frank et al., 1997; *Polistes* wasps, Starks et al., 1998b; colonial marine hydrozoans, Wilson and Grosberg, 2004).

In social insect societies, where individuals often form large and complex colonies, nestmate recognition is essential for maintaining colony integrity. It has long been recognized that workers are able to discriminate between nestmates and non-nestmates or, more accurately, between colony-mates and noncolony-mates (reviewed in: Gamboa et al., 1986; Breed and Bennett, 1987; Vander Meer and Morel, 1998), however more recent studies indicate that workers are also able to vary the intensity of their response towards non-nestmates under specific circumstances. For example, repeated contact with a neighbouring colony can lead to a reduction in aggression towards neighbouring workers (Gordon, 1989; Dunn and Messier, 1999; Thomas et al., 1999; Langen et al., 2000). Diminished aggression between neighbours relative to strangers has also been observed in a variety of taxa including territorial mammals (e.g. Barash, 1974), birds (Molles and Verencamp, 2001), amphibians (Jaeger, 1981), reptiles (Qualls and Jaeger, 1991) and fish (e.g. Leiser, 2003). This “dear enemy phenomenon” suggests that familiarity with the cues of one’s neighbours allows a territorial individual to reduce some of the costs associated with territory defense – neighbours are recognized and tolerated while foreign individuals that may be encroaching on one’s territory are not. However, the opposite pattern can also be observed; frequent contact with competitors can lead to recurrent or higher levels of aggression between neighbours (Jutsum et al., 1979; Sanada-Morimura et al., 2003; Velasquez et al., 2006). Ultimately, the expression of aggression arises from an interplay among several factors, including the relatedness between the interacting individuals, the developmental history of the individuals involved, the social context within which the interaction takes place, and the fitness consequences of the interactions.

In this study we investigate genetic and context-dependent aspects of nestmate recognition in the highly polydomous Argentine ant (*Linepithema humile*). The Argentine ant has become a focal system in nestmate recognition studies, in part because reduced intraspecific aggression typical of introduced populations is thought to contribute to its widespread success as an invader (Holway et al., 1998). The absence of behavioural borders among physically separate nests is typical of unicolonial populations, and common among populations of introduced ants (Hölldobler and Wilson, 1977; Passera, 1994; Holway et al., 2002). Intraspecific aggression in Argentine ants may be influenced by both genetically based recognition cues (Tsutsui et al., 2000; Suarez et al., 2002) and cues derived from the environment (Chen and

Nonacs, 2000; Liang and Silverman, 2000). However, as with social insects generally, certain aspects of the Argentine ant’s nestmate recognition system also exhibit context dependency (Suarez et al., 2002; Roulston et al., 2003; Buczkowski and Silverman, 2005; Thomas et al., 2005a, 2006).

One potential example of context-dependent nestmate recognition in the Argentine ant relates to a worker’s experience with conspecific supercolonies. Thomas et al. (2005, 2006) reported that workers close to territory borders (i.e. neighbours) exhibited higher levels of aggression towards conspecifics than did workers collected 500 m from territory borders (i.e. non-neighbours). These data suggest that contact between supercolonies reinforces elevated levels of aggression; workers from nests in the center of a supercolony will have little to no experience with aggressive conspecifics because all neighbouring nests within a supercolony consist of workers from the same supercolony, but workers from nests near territory borders will regularly encounter aggressive conspecifics. However, this pattern might also arise from differences in genetic similarity between neighbours and between non-neighbours, because genetically based recognition cues appear to be involved in intraspecific aggression in Argentine ants (Tsutsui et al., 2000; Suarez et al., 2002). Furthermore, high aggression among neighbours may change temporally. For example, the frequency of neighbour-neighbour interactions may well change with seasonal fluctuations in worker abundance or environmental variables. In southern California where worker populations are near their annual low in spring and near their annual maximum in late summer (Markin, 1970), we would predict that neighbour-neighbour interactions would intensify over this time span and that aggression might increase as a result.

In this study we use Argentine ants to examine the context-dependent nature of nestmate recognition. Unlike other behavioural studies on this species, we focus on aggression levels at naturally occurring territory boundaries. Because direct interactions at territory boundaries will influence the costs of territoriality and the likelihood that colonies will exchange genes, focusing on the behaviour of established and opposing supercolonies is necessary to advance an understanding of context-dependent nestmate recognition. To discriminate between experiential and genetic causes of elevated aggression, we contrast aggression levels and patterns of genetic variation between neighbours (i.e., workers from nests located in the immediate vicinity of territory borders that have the opportunity to interact daily) and between non-neighbours (i.e., workers from nests located far enough away from a territory border so that interactions are unlikely). In addition, we assess the extent to which aggression between neighbours and between non-neighbours changes seasonally. We compliment our field study with a laboratory experiment to test how the degree of worker experience with opposing supercolonies affects levels of aggression. Taken together these complimentary

approaches provide a detailed examination of the factors that influence variation in the nestmate recognition system of a widespread and abundant introduced ant species.

Methods

Study system

Five supercolonies of Argentine ants are currently known to occur in southern California: a large supercolony (LC) that extends as a non-aggressive behavioural unit throughout much of California (Tsutsui et al., 2000) and four spatially restricted supercolonies: Lake Hodges (LH), Sweetwater (SW), Lake Skinner (LS) (Suarez et al., 2002; Tsutsui et al., 2003), and Cottonwood (CW). These four smaller supercolonies are not known to contact one another, but all of them (except for LS) directly abut portions of LC. At present LC and LS are separated by a 400-m buffer of dry ground that is inhospitable to *L. humile*. Based on present knowledge concerning the smaller supercolonies, LH occupies a larger area compared to the area occupied by SW, LS or CW and for this reason we were able to examine interactions between LH and LC in the most detail (for map see Suarez et al., 2002). Using territory boundaries described in companion papers (Thomas et al., 2005a, 2006) as reference locations, we sampled Argentine ant workers along transects running perpendicular to these territory boundaries to investigate factors influencing the differing levels of intraspecific aggression between neighbours and between non-neighbours.

Seasonal variation in aggression between neighbours and between non-neighbours

To assess the extent to which aggression between neighbours and between non-neighbours changes over time, we collected workers for behavioural assays from 16 different territorial boundaries at monthly intervals from April to September 2004. Twelve of the 16 territory borders were between LH and LC and two were between SW and LC, and two between CW and LC. All collection sites were ≥ 200 m apart. At each boundary, we collected foraging neighbours and non-neighbours. Neighbours were collected within 1 m from territory borders, whereas non-neighbours were collected away from territory boundaries (500 m from boundaries for LC and LH, and ~ 150 m for SW and CW). Distance differences for non-neighbours reflect the unequal sizes of the LH, SW and CW supercolonies. To quantify aggression levels we conducted two sets of behavioural assays: (1) between neighbours (workers from nests located in the immediate vicinity of territory borders that have the opportunity to interact daily), and (2) between non-neighbours (workers from nests located far enough away from a territory border so that interactions are unlikely). Assays were undertaken in the laboratory immediately after collection. For each combination of territory boundary and distance pairings we conducted four bioassays and used the mean of these replicated assays in all analyses.

Behavioural assays

In all cases in which we quantified aggression, we used five-on-five behavioural assays modified from Roulston et al. (2003) (see Thomas et al., 2005b). Five ants from each supercolony were placed in a 10 cm² diameter petri dish lined with fluocon to prevent ants from escaping. We scanned worker behaviours every minute for 10 minutes and classified observed interactions into one of four categories: (1) no apparent aggression (e.g., workers either ignore one another or interact as would nestmates), (2) mandible gaping, avoidance (e.g., workers make contact and at least one or the other then recoils or runs away), or prolonged antennation (e.g., workers closely inspect one another with their antennae), (3) overt aggression (e.g., a physical attack by one or both workers, including lunging, biting, holding or pulling of legs or antennae), and (4) intense fighting (e.g., aggression resulting in death

or severe injury – can include the use of chemicals). To obtain a single aggression index, we first determined the proportion of ants involved in each behaviour category at every minute increment. We then multiplied this proportion by the aggression level for that behaviour (i.e. 1–4), and the sum of these numbers gave the final aggression score (see Thomas et al., 2005b, 2006). A score of 1 indicates no aggression throughout the ten scans, while a score of 4 indicates that all ants were fighting at the highest level within the first minute. Observers were blind to the identity of supercolony pairings and whether trials involved neighbours or non-neighbours. For data analyses each supercolony pair was considered separately.

Intraspecific aggression and genetic similarity between neighbours and between non-neighbours

At six of the LC-LH territory borders described under “seasonal variation in aggression between neighbours and between non-neighbours” we collected workers for genetic analysis from nests that were 1 m (neighbours) and 500 m (non-neighbours) on either side of the territory border. We purified DNA from ten workers per nest and genotyped each individual using four polymorphic microsatellite markers, following the procedures described in Thomas et al. (2006). Previous studies using these loci have shown that they are sufficient to detect colony boundaries, when such boundaries occur (Thomas et al., 2006). Specifically, our analyses using these four loci in the program Structure (Pritchard et al., 2000), which implements Bayesian assignment tests, correctly assigned virtually all individuals to their colony of origin, without using *a priori* information regarding colony identity. Thus, we are confident that we can use these loci to detect differences in genetic similarity and relatedness between the nests used in this study. We calculated pairwise relatedness values using the program Relatedness 5.0 (Queller and Goodnight, 1989) and the average percent alleles shared between pairs of nests. In particular, we wished to determine if there was a difference in the genetic similarity between non-neighbours and between neighbours. If workers that show lower levels of aggression are more genetically similar than workers that are aggressive toward each other, then we cannot reject the hypothesis that the observed behavioural variation simply reflects underlying genetic relationships. Alternatively, this hypothesis can be rejected if more aggressive workers are more genetically similar to each other, or if there is no correspondence between aggression and behavioural patterns.

Laboratory experiment: a manipulation of worker experience

Behavioural and genetic comparisons between neighbours and between non-neighbours provide important information concerning the potential influences of worker experience and genetic similarity on levels of aggression. From these comparisons, however, it is not possible to manipulate different degrees of worker experience or to examine the time period over which such experience may be acquired. For these reasons, we conducted a lab experiment in which we paired aggressive colonies together and controlled the extent to which workers from each colony could interact with one another. We reared experimental colonies under three different levels of worker contact: (1) unrestricted exposure (i.e., unlimited worker interchange between aggressive colonies), (2) restricted exposure (i.e., workers from aggressive colonies could antennate one another through a mesh barrier but no worker interchange was allowed), and (3) no exposure (e.g., pairs of aggressive supercolonies do not contact one another).

The experimental design of this lab study reflects the somewhat unusual nature of the introduced population of Argentine ants in California (Tsutsui et al., 2000; Suarez et al., 2002; see Giraud et al., 2002, for a description of a similar pattern for the introduced population in southern Europe). Each experimental group had ten pairs of experimental colonies, and the pairings in each group consisted of unique pairwise combinations of the five supercolonies currently known from southern California (Suarez et al., 2002; Thomas et al., 2006). Experimental colonies originating from LH, for example, were paired against experimental colonies from SW, CW, LS and LC but not against other LH colonies. (See Holway et al. (1998) and Holway and

Suarez (2004) for further examples and discussions of this type of experimental design). The pairings in each of the three experimental groups were identical. Note that we ran separate statistical tests on each experimental group individually because our primary goal was to examine how worker experience affects aggression over time rather than to compare levels of aggression among experimental groups.

Aggressive colonies within each pair had no known history of contact. Material for experimental colonies originated from sites near the center of LH (> 1 km from its border with LC), SW (~ 150 m from its border with LC), CW (~ 700 m from its border with LC) and LS (~ 500 m away from the closest point with LC). We collected samples from LC > 10 km away from any known territory boundary. For LH, CW, SW, and LC, we collected colony material from four different sites, each separated by at least 200 m. For LS, the smallest of the five supercolonies, we collected colony material from two sites separated by 200 m. Within each of the three experimental groups, representatives from the five supercolonies were each represented in four of the ten pairings, but each of those four experimental colonies originated from a different collecting site within that particular supercolony (except for LS as noted).

Each experimental colony consisted of 2 queens and approximately 1000 workers and 100 pieces of brood. Experimental colony fragments were housed in circular plastic containers (20 cm in diameter) lined with flunon to prevent ants from escaping. Each nest container was provided with nesting chambers consisting of test tubes covered with aluminium foil, half filled with water and plugged with cotton wool. We fed experimental colonies sugar water daily and *Drosophila* and egg once a week. Experimental colonies were reared at a constant 25°C and left for five days prior to the start of the experiment. Each nest container was fitted with a plugged, one-meter exit tube (6 mm in diameter). In the unrestricted exposure treatment we joined pairs of experimental colonies together by fitting their exit tubes with a plastic connector, which was in turn connected to a larger piece of tubing (15 mm in diameter and 20 cm in length) that linked colonies together. This treatment allowed workers to contact and fight with the opposing supercolony. The restricted exposure treatment resembled that of the unrestricted exposure treatment except that we placed a barrier consisting of two pieces of fine mesh at the midpoint of the connecting tube that joined experimental colonies. Workers in the restricted exposure treatment could, at most, antennate workers from the other supercolony. In the zero exposure treatment, pairs of experimental colonies remained unconnected. In the two connected treatments we maintained connections for 4 weeks, and then severed all connections but continued to rear all experimental colonies for another 4 weeks.

We performed behavioural assays (as above) prior to the connection of colonies (week 0), four weeks after colony pairs were first connected (week 4), and four weeks after disconnection (week 8). To test for changes in aggression levels over time, we used multivariate repeated-measures analysis of variance (MANOVA). We ran a separate MANOVA for each experimental group. Following MANOVAs, we used single degree-of-freedom polynomial (linear and quadratic) contrasts, as recommended by Gurevitch and Chester (1986) to test null hypotheses. Linear contrasts test the hypothesis that aggression levels at the start of the experiment (week 0) differ from aggression levels at the end of the experiment (week 8), whereas quadratic contrasts test the hypothesis that aggression levels in the middle of the experiment (week 4) differ from the mean of the aggression levels between the beginning and the end of the experiment (week 0 and week 8).

Results

Seasonal variation in aggression between neighbours and between non-neighbours

Overall, ants from different supercolonies displayed high levels of aggression toward each other, as has been reported in numerous previous studies (e.g., Suarez et al.,

1999; Tsutsui et al., 2000, 2003; Tsutsui and Case, 2001; Giraud et al., 2002; Buczkowski and Silverman, 2005; Thomas et al., 2006). However, neighbours consistently displayed higher levels of aggression than did non-neighbours in all LC-LH pairings (Fig. 1) (Repeated-measures ANOVA: Treatment $F_{1,22} = 268.26$, $P < 0.001$; Month $F_{5,110} = 6.49$, $P < 0.001$; interaction $F_{5,110} = 10.19$, $P < 0.001$). Because of the significant interaction term between month and treatment (neighbour versus non-neighbour), we have analyzed the two treatment categories separately. For non-neighbours, levels of aggression varied over time (Fig. 1) (Repeated-measures ANOVA: $F_{5,55} = 11.32$, $p < 0.001$), but comparisons involving neighbours revealed a consistently high level of aggression over the six-month period (Repeated-measures ANOVA: $F_{5,55} = 1.13$, $p = 0.355$).

Because of the small size of the SW and CW supercolonies, we were only able to monitor two sites that contacted LC in each. Despite these small sample sizes, a similar pattern to that observed at the LH-LC border emerged; neighbours appeared to react more aggressively towards each other than did non-neighbours at 3 out of 4 of these sites (Fig. 1b, 1c). At one site (CW-LC), however, neighbours and non-neighbours displayed similar levels of aggression (Fig. 1c).

Intraspecific aggression and genetic similarity between neighbours and between non-neighbours

The disparity in aggression between neighbours and between non-neighbours cannot be explained by differences in relatedness or genetic similarity. The average pairwise relatedness between workers from nests 1 m on either side of the colony boundaries (neighbours) did not significantly differ from the pairwise relatedness between workers from pairs of nests 500 m from the colony boundary (non-neighbours) (Table 1). When we calculated genetic similarity using percent alleles shared, there was a marginally significant difference between neighbours and between non-neighbours, but in the opposite direction than that expected based on levels of aggression (Table 1). That is, neighbours were slightly more genetically similar to one another compared to non-neighbours, but neighbours were also more aggressive towards each other.

Laboratory experiment: a manipulation of worker experience

When experimental colonies of Argentine ants were provided with at least some level of contact with another supercolony, aggression levels increased after 4 weeks of exposure, whereas experimental colonies in the no exposure treatment did not exhibit any significant change in aggression over time (Fig. 2, Table 2). Elevated levels of aggression resulting from contact proved short lived,

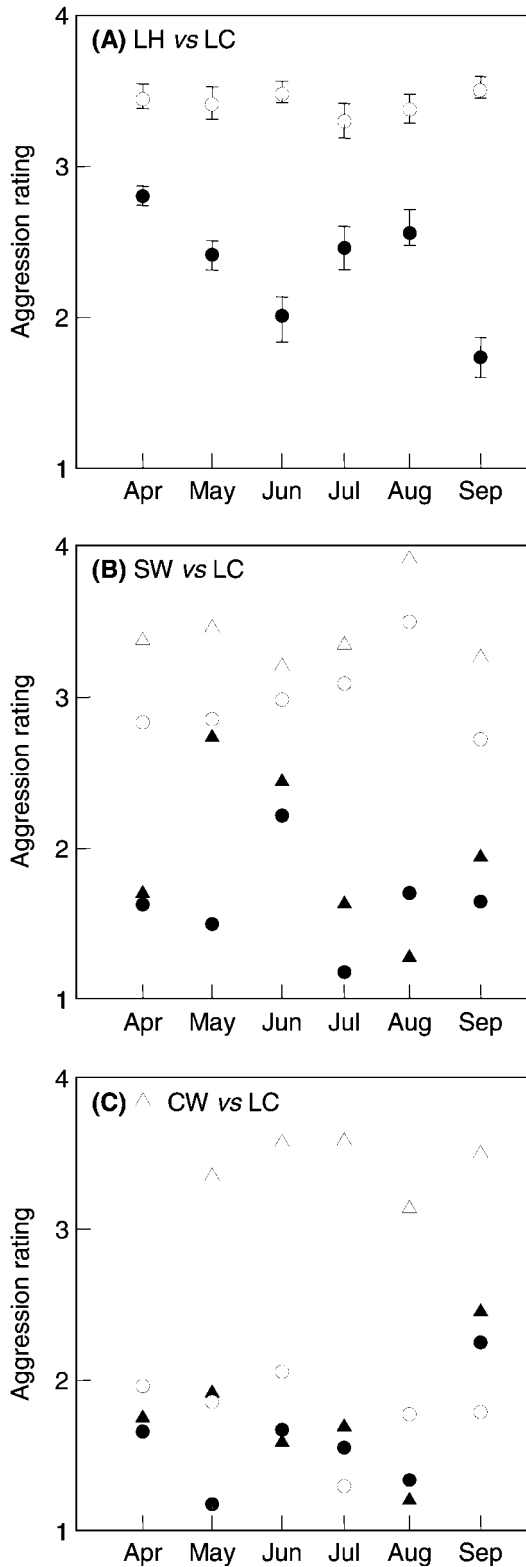


Figure 1. Aggression levels for monthly field bioassays between neighbours (○) and between non-neighbours (●). (A) The Lake Hodges supercolony (LH) versus the large supercolony (LC) (n = 12), (B) the Sweetwater supercolony (SW) versus LC (n = 2), (C) the Cottonwood supercolony (CW) versus LC (n = 2). For (A), the mean (± SE) levels of aggression are shown for the 12 transects. For (B) and (C) individual transects are illustrated by different symbols (triangles and circles).

decreasing in all replicates after connections between supercolonies were severed (Fig. 2). Significant quadratic contrasts in the unrestricted and restricted exposure treatments indicate that levels of aggression were higher when supercolonies were in contact with one another (week 4) than when such connections were lacking (week 0 and week 8; Table 2). Although aggression levels decreased in the unrestricted and restricted exposure treatments after colony connections were broken (Fig. 2; between weeks 4 and 8), significant linear contrasts indicate that aggression levels at the end of the experiment had not quite declined to levels observed at the beginning of the experiment (Fig. 2, Table 2).

Discussion

Although there is abundant evidence from social insects that experiences early in adult life (soon after eclosion) can affect recognition behaviour later in life (reviewed in: Breed, 1983; Gamboa et al., 1986; Breed and Bennett, 1987; Vander Meer and Morel, 1998), fewer studies have examined behavioural plasticity in older individuals. However, because most organisms live in fluctuating environments – in terms of resource availability, competition, abiotic factors, and innumerable other variables – it is easy to imagine how such behavioural plasticity could be adaptive and advantageous. Here, we provide evidence that workers of the invasive Argentine ant respond to interactions with non-colonymates by elevating levels of aggression displayed toward ants from foreign colonies. This heightened aggression between neighbours does not vary seasonally, nor does it arise from greater genetic differentiation at territory borders, but it is directly related to the history of contact between conspecifics.

Evidence that the level of intraspecific aggression between supercolonies is specifically related to the history of contact between supercolonies comes both from field observations and laboratory experiments. In the field, the consistently high levels of aggression between neighbours observed over the six-month study period (Fig. 1) suggest that elevated aggression can be triggered by the mere presence of aggressive conspecifics. The lab experiment supports this notion in that only minimal levels of contact between supercolonies are necessary to elevate aggression between workers. When we connected experimental colonies from different supercolonies with no history of contact in the lab, aggression significantly increased (Fig. 2). Elevated aggression was also observed when contact between aggressive supercolonies was restricted to workers antennating one another through a mesh barrier. However, when connections between laboratory colonies were severed, aggression declined suggesting that repeated exposure to appropriate stimuli is important for maintaining high aggression levels.

Table 1. Genetic similarity of neighbouring and non-neighbouring nests belonging to different supercolonies.

Transect	<i>Relatedness</i>		<i>p</i>	Percent alleles shared		<i>p</i>
	Neighbouring	Non-neighbouring		Neighbouring	Non-neighbouring	
1	0.118	0.021		0.222	0.371	
2	-0.050	-0.071		0.273	0.446	
3	-0.042	-0.190		0.304	0.223	
4	-0.124	0.088		0.344	0.452	
5	-0.249	0.027		0.290	0.389	
6	-0.243	0.091		0.460	0.611	
Mean	-0.098	-0.025	0.322	0.316	0.416	0.046
S.D.	0.139	0.108		0.081	0.127	

Table 2. Results of the lab experiment that tests how different degrees of exposure affect levels of aggression between Argentine ants from different supercolonies. Repeated measures MANOVA was performed on aggression data before, during and after connections between supercolonies were manipulated. Single-degree-of-freedom polynomial contrasts were run to test null hypotheses.

Treatment	λ	MANOVA results				Source	SS	Polynomial contrasts				Source	SS	df	Quadratic ²	
		Wilk's F	df	P	df			F	P	F	P					
Unrestricted	0.09	39.71	2,8	<0.001	Time	0.36	1	16.33	<0.005	Time	4.31	1	53.36	<0.001		
					Error	0.20	9			Error	0.73	9				
Restricted	0.39	6.31	2,8	<0.05	Time	0.43	1	7.39	<0.05	Time	1.46	1	14.20	<0.005		
					Error	0.53	9			Error	0.93	9				
No exposure	0.90	0.44	2,8	0.66	Time	0.04	1	0.45	>0.05	Time	0.07	1	0.47	>0.05		
					Error	0.73	9			Error	1.34	9				

¹Tests the hypothesis that aggression levels at the start of the experiment (0 weeks) differ from aggression levels at the end of the experiment (8 weeks).

²Tests the hypothesis that aggression levels in the middle of the experiment (4 weeks) differ from the mean of the aggression levels between the beginning and the end of the experiment (0 and 8 weeks).

The genetic relationships between neighbours and between non-neighbours provide additional support that heightened aggression at colony boundaries results from prior behavioural interactions rather than subtle differences in genetic similarity. If the level of aggression displayed by Argentine ants was solely a function of kinship, one would expect a negative relationship between genetic similarity and mean aggression score. Instead, our genetic analyses showed that neighbours were not significantly less closely related to each other compared to non-neighbours (Table 1). Moreover, when percent alleles shared is used as the measure of genetic similarity, neighbours are slightly more similar to one another than are non-neighbours (Table 1), a pattern opposite of that expected if heightened aggression was due to greater genetic dissimilarity. These data also suggest that there is some degree of local gene flow across some of these colony boundaries, as reported in Thomas et al. (2006), but that the resulting increase in genetic similarity at colony boundaries does not offset the heightened aggression caused by previous and repeated encounters between neighbours. Other studies on Argentine ants have reported similar abrupt colony boundaries with little gene flow (Jaquiere et al., 2005;

Pedersen et al., 2006; Thomas et al., 2006) indicating that these results are likely to apply generally for this species.

Although we provide evidence that the recognition system of Argentine ants is influenced by the history of contact between conspecifics, it is critical to note that these findings do not contradict previous findings in this system of an overall relationship between genetic similarity and aggression (Tsutsui et al., 2000; Thomas et al., 2006). Instead, genetic relationships appear to dictate the basal level of aggression displayed between naive Argentine ants (i.e., non-neighbours), but interactions with conspecifics at territory boundaries can heighten levels of aggression. In other words, aggression changes from high to very high, depending on the history of interactions. In all cases workers from different supercolonies fought aggressively with one another.

Our results, although not in agreement with the dear enemy phenomenon, do illustrate the context-dependent nature of elevated aggression (see also Thomas et al. 2005a). In other circumstances where dear-enemy does not occur, heightened aggression between neighbours has been explained by competition for territorial resources (e.g. Leiser, 2003), or frequent movement of

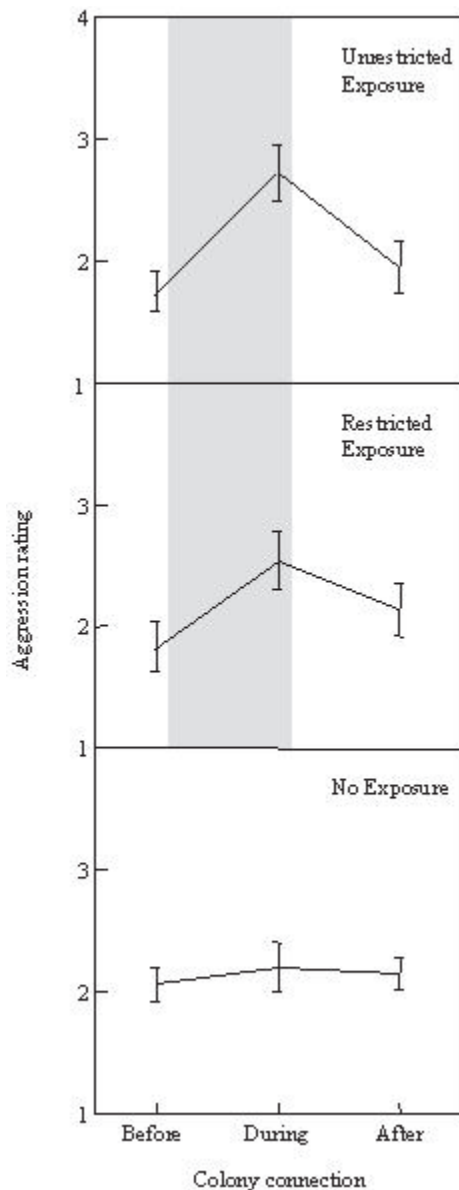


Figure 2. Mean (\pm SE) aggression levels between experimental colonies of Argentine ants before (week 0), during (week 4) and after (week 8) colony connection. Shaded portions of the graphs for the “unrestricted” and “restricted” exposure treatments indicate the interval (0–4 weeks) when experimental colonies in these treatment groups were connected in pairs. Experimental colonies making up each replicate in the “no exposure” treatment remained separate for the entire experiment.

nest sites (Sanada-Morimura et al., 2003). In Argentine ants, when aggressive conspecifics are present, there is a real risk of costly territorial conflict (Thomas et al., 2006). Maintaining elevated aggression levels at territory borders could be ecologically advantageous to colonies, because workers that initiate aggression are more likely to survive one-on-one interactions (Tsutsui et al., 2003). Therefore workers that are on “red alert” (i.e., show elevated aggression) might result in a higher likelihood of quelling costly territorial battles before they start or of

winning such conflicts when they do erupt. In contrast ants living far from colony boundaries (non-neighbours), may never contact aggressive conspecifics and presumably benefit from having less elevated levels of aggression. A slightly relaxed recognition threshold might result in timesavings when workers from the same colony contact one another, and could thus improve colony efficiency. Investigating this phenomenon in more detail in native populations of Argentine ants would provide valuable information concerning why workers might benefit from being more aggressive towards neighbours than non-neighbours.

The plasticity (or learned behaviour) of the Argentine ants recognition system reported here may have been shaped by the somewhat unusual colony structure and nesting behaviour of this species. Because Argentine ants exhibit semi-nomadic behaviour and frequently relocate their nests (Newell and Barber, 1913; Markin, 1970), the ability to recognize new neighbours and forget former ones may be a beneficial strategy. Sanada-Morimura et al. (2003) argue a similar point for the ant *Pristomyrmex pungens*, which inhabits ephemeral nest sites and possesses a nestmate recognition system with similarities to the one documented here for *L. humile*. In both species a continual input of relevant stimuli is required to maintain the higher levels of aggression that neighbours display towards one another compared to the level of aggression evident among non-neighbours. Future work could focus on the specificity of the stimulus required to elevate aggression in response to neighbours. For example, do colonies recognize specific odors of an aggressive neighbour or do all aggressive neighbours elicit the same response? Does neighbour recognition apply to heterospecifics as well as to conspecifics? Answers to these questions seem readily amenable to experimental analysis and would add to the growing understanding of how ecological circumstances contribute to variation in nestmate recognition systems generally.

Our results are consistent with previous work suggesting that the threshold for the expression of aggression among colonies has a genetic basis in Argentine ants (Tsutsui et al., 2003; Thomas et al., 2006). However, these findings also illustrate that interactions between non-nestmates, such as the history of contact and the circumstances surrounding interactions, can alter the intensity of aggression. The dual inputs of both genetic and behavioural factors in the recognition system of Argentine ants are consistent with the optimal acceptance threshold model (Reeve, 1989), as well as empirical data from several other self/non-self recognition systems (Starks et al., 1998b). For example, in the marine hydrozoan, *Hydractinia symbiolongicarpus*, colonies compete for space on shells of hermit crabs, *Pagurus longicarpus*. As in Argentine ants and other social insects, the genetic relationships between *Hydractinia* colonies play a central role in whether colonies fuse or aggressively reject each other (Yund et al., 1987; Grosberg et al., 1996; Mokady and Buss, 1996; Wilson and Grosberg, 2004), but fre-

quencies of fusion events also decline through time, in tandem with decreasing encounter frequencies between colonies (Wilson and Grosberg, 2004). Similarly, the expression of nestmate recognition appears to be context-dependent in the wasp, *Polistes dominulus* (Starks et al., 1998b). Discrimination occurs when *Polistes* workers encounter a foreign individual in the context of their own nest, specifically when the presence of a familiar nest fragment or a nestmate indicates that the colony is nearby. The context-dependent expression of recognition behaviours documented for these and other taxa suggest that plasticity in self/non-self recognition is taxonomically widespread, and may be a more general phenomenon than is currently appreciated.

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