

Intraspecific competition influences the symmetry and intensity of aggression in the Argentine ant

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Cooperative social groups rely on the ability to distinguish members from nonmembers. Accordingly, social insects have evolved a variety of systems that allow discrimination of nest mates from non-nest mates. In this study, we show that experience can modify patterns of intraspecific aggression in Argentine ants (*Linepithema humile*). In laboratory experiments, we found that aggression between colonies was often asymmetrical, but in all five cases, this asymmetry shifted to symmetrical aggression after contact with a hostile colony. Moreover, in the field, aggression between workers collected from colony borders was symmetrical, whereas polarized aggression occurred between workers collected 500 m away from colony borders. Coinciding with this shift in aggression symmetry, we also observed an increase in both the overall level of aggression and the frequency of aggression in both the field and laboratory bioassays. We found little evidence for colony-level competitive asymmetries stemming from polarities in aggression at the worker level, either in the laboratory or in the field. These results illustrate that recognition systems in Argentine ants are surprisingly dynamic and provide experimental evidence for how recognition can be adjusted in response to specific circumstances—in this case the presence of intraspecific competitors. *Key words*: aggression symmetry, intraspecific competition, invasive species, nest-mate recognition, uniclonality. [*Behav Ecol* 16:472–481 (2005)]

The ability of organisms to distinguish between self and nonself is a fundamental organizing principle in biology. The recognition systems that underlie this discrimination have played a critical role in several of the major transitions during the evolution of life: the fusion of prokaryotes into eukaryotes, the aggregation of single-celled organisms into multicellular organisms, and the formation of colonial social groups from independent individuals (Buss, 1987; Grosberg, 1988; Maynard-Smith and Szathmáry, 1995; Queller, 2000; Queller and Strassmann, 2002). Accordingly, the breakdown of recognition systems can have negative consequences such as the expression of autoimmune disorders, the evolution of social parasitism, and the transition of social organisms into damaging invasive species (Buss, 1987; Janeway and Travers, 1997; Sherman et al., 1997; Tsutsui and Suarez, 2003).

Social insects, by definition, live within closed societies. The integrity of these groups is maintained by the ability of group members to distinguish colony mates (self) from members of foreign colonies (nonself). There is an abundance of evidence that young adults imprint on specific characters that later allow them to distinguish colony members from nonmembers (Breed and Bennett, 1987; Hölldobler and Wilson, 1990; Sherman et al., 1997). In ants, for example, it is believed that newly eclosed workers imprint on the odors of ants around them (typically colony mates) and use this reference (or template) as a standard. Other individuals are compared against this template for the remainder of the ant's life span (Breed and Bennett, 1987; Hölldobler and Wilson, 1990; Sherman et al., 1997).

However, social insect recognition systems can also be dynamic as they are modulated in response to experiences

later in life. In some cases, repeated contacts with individuals from neighboring (presumably competing) colonies can lead to habituation, manifested as a reduction in overt aggression toward ants from neighboring colonies (Jutsum et al., 1979; Langen et al., 2000; Thomas et al., 1999). This “dear-enemy” phenomenon is believed to reduce costs associated with frequent fights between neighbors (Ydenberg et al., 1988). Conversely, repeated contacts with competitors can lead to increased aggression toward individuals from previously encountered colonies (Dunn and Messier, 1999; Gordon, 1989; Sanada-Morimura et al., 2003). In these cases, it appears that frequent contacts with individuals from foreign colonies cause increased sensitivity to these individuals, resulting in more frequent or higher levels of aggression. This phenomenon might occur in environments with variable resources where competition is high between neighboring colonies, but little competition occurs between distant colonies (Sanada-Morimura et al., 2003).

In this study, we address the roles of experience and competition as factors influencing patterns of intraspecific aggression in the invasive Argentine ant (*Linepithema humile*). Argentine ants in their native range typically display aggression at short spatial scales, nearly always reacting aggressively to conspecifics that come from nests more than a kilometer away (Suarez et al., 1999; Tsutsui et al., 2000). In contrast, workers from introduced populations treat practically all conspecifics as nest mates (Markin, 1968; Newell and Barber, 1913; Suarez et al., 1999; Way et al., 1997) and form expansive supercolonies that can occupy hundreds of thousands of hectares (Giraud et al., 2002; Tsutsui et al., 2000; but see Buczkowski et al., 2004). This form of social organization, called uniclonality (Hölldobler and Wilson, 1990), may reduce costs associated with intraspecific competition and territoriality (Holway and Suarez, 2004; Holway et al., 1998; Horton, 1918).

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Previous studies indicate that extreme unicoloniality may have arisen in introduced populations of Argentine ants after the loss of genetic diversity during introduction (Tsutsui and Suarez, 2003; Tsutsui et al., 2000). This loss of genetic diversity increased levels of genetic similarity in introduced populations (Tsutsui and Case, 2001), which in turn may have led to the widespread cooperation that characterizes unicoloniality (Tsutsui et al., 2000). Genetic diversity may have declined even further because workers from colonies with low levels of genetic diversity preferentially attack and kill workers from more diverse colonies (Tsutsui et al., 2003). This hypothesis has been tested on the Californian populations of Argentine ants, which currently consist of one large supercolony that occurs in many parts of coastal California, and three smaller spatially restricted supercolonies. The large supercolony is genetically less diverse than the three smaller supercolonies, and in one-on-one aggressive encounters, workers from the large supercolony initiate aggression more often (Tsutsui et al., 2003). Because attackers survive fights more than six times as often as recipients of aggression, the costs associated with asymmetrical aggression appear to be large (Tsutsui et al., 2003). However, it is not known if these costs at the individual level translate directly into differences in competitive ability at the colony level.

Here we use a complementary set of experimental approaches to test (1) what role experience plays in altering the intensity and polarization of intraspecific aggression at both the worker and colony levels and (2) whether worker-level asymmetries in intraspecific aggression translate into disparities in competitive performance at the colony level. By expanding on previous work conducted at the individual level, the colony-level experiments described here aim to illuminate the mechanisms underlying nest-mate recognition in Argentine ants, in particular, how these mechanisms may have led to the formation and maintenance of the extreme unicoloniality that characterizes introduced populations of the Argentine ants (e.g., Giraud et al., 2002; Tsutsui et al., 2000, 2003) and introduced populations of other invasive ants (Holway et al., 2002; Le Breton et al., 2004; Passera, 1994; Tsutsui and Suarez, 2003). More generally, this study experimentally tests how experience can affect patterns of nest-mate recognition in social insects and how changes induced by these experiences may influence the outcome of competitive interactions among colonies.

METHODS

Laboratory experiments

Nest collection and maintenance

To test for asymmetries in competitive ability and to determine what role experience plays in altering the symmetry of intraspecific aggression, we paired equal-sized colony fragments from nine different sites. Six of these sites were occupied by the large supercolony (designated by subscript L: Los Peñasquitos [LP_L], La Jolla [LJ_L], San Luis Rey River [SL_L], Santa Margarita River [SM_L], Tijuana River [TJ_L], and University Town Center [UT_L]). The remaining three sites are currently occupied by three separate smaller supercolonies (Suarez et al., 2002) (designated by subscripts S1, S2, or S3: Lake Hodges [LH_{S1}], Lake Skinner [LS_{S2}], and Sweetwater [SW_{S3}]). All collection sites, irrespective of colony identity, were separated by at least 10 km. Previous work has shown that aggression between the supercolonies used in this study is polarized (Tsutsui et al., 2003). Workers from the large supercolony and SW_{S3} always act as the aggressors in pairings with other supercolonies; no polarization is evident between the large supercolony and SW_{S3}.

We divided colony fragments into a total of 42 experimental subcolonies, each containing four queens and approximately 1000 workers and 100 pieces of brood. We made three experimental subcolonies from each of the six large supercolony sites (total, 18) and eight subcolonies from each of the three smaller supercolony sites (total, 24). As controls, we maintained one subcolony from each site in isolation.

We used two groups of experimental subcolony pairs: (1) large supercolony versus small supercolony pairings and (2) small supercolony pairings. Group 1 pairs consisted of one experimental subcolony from the large supercolony and one from a smaller supercolony, resulting in a total of 18 experimental pairs. Group 2 pairs consisted of only small supercolony pairings (LH_{S1} versus LS_{S2}, SW_{S3} versus LH_{S1}, and SW_{S3} versus LS_{S2}) replicated twice, resulting in a total of six experimental pairs. Both experimental subcolony groups were used to investigate worker-level aggression; however, experimental subcolonies from Group 1 were also used to determine colony-level competition. Using only the large supercolony versus small supercolony pairings to determine colony-level competition incorporates an important element of realism because all of the smaller supercolonies are currently surrounded by the large supercolony and do not share a common boundary with another small supercolony.

Experimental subcolonies were housed in identical circular plastic containers (20 cm in diameter) lined with Fluon™ to prevent ants from escaping. The nesting chamber differed between the two groups of experimental subcolonies. In the large supercolony versus small supercolony pairs, the floor of each container was covered with plaster. Two petri dishes 10 cm in diameter were placed in each container to serve as nest chambers. Using silicon, these petri dishes were sealed to the plaster but elevated above it by 2 mm. There was one entrance to each petri dish. Water was added to the edge of the plaster daily to keep the nests moist. We gave these subcolonies water ad libitum using a small plastic dish with cotton wool. Using this form of nest chamber enabled us to count the number of workers and brood in each subcolony without disturbing the nest. Experimental subcolonies used in the small supercolony pairings were housed using nesting chambers consisting of test tubes half filled with water and plugged with cotton wool. Both subcolony groups were kept at a constant 25°C and fed crickets once per week and 25% sugar water daily.

We gave subcolonies 10 days to adjust to laboratory conditions and then connected pairs to a common foraging arena (a circular plastic container 20 cm in diameter) made accessible to each subcolony via a 3-m piece of plastic tubing. After the subcolonies were connected, food was placed only in the common foraging arena, but each subcolony had access to water in its nesting container.

Colony-level competition—large supercolony versus small supercolony pairs

We measured colony-level competitive performance in four different ways: number of living workers, brood production, foraging activity, and resource consumption. Foraging activity was measured as the number of days each colony was observed foraging in the common arena 1 h after we placed sugar water in the arena. Each week, we measured resource consumption by introducing six freshly killed adult *Drosophila* into the foraging arena. At 20-min intervals, over a 2-h period, we recorded the number of *Drosophila* retrieved by each subcolony and the number remaining within the foraging arena. The subcolonies were not fed for 2 days prior to collection of data on resource consumption. We counted the number of living workers at three time intervals (5, 10, and 14 weeks) and

the amount of brood present at the end of the 14-week observation period.

For data analyses, we combined the 18 subcolony pairs into three groups corresponding to each type of pairing (large supercolony versus LH_{S1}, large supercolony versus LS_{S2}, or large supercolony versus SW_{S3}). Each subcolony pair was considered a replicate. We used one-sample *t* tests to test for differences in the number of brood and living workers between subcolonies at the end of the 14-week period. Foraging activity and resource consumption data were analyzed using paired *t* tests.

Worker-level aggression—all supercolony pairings

To determine what role experience plays in altering the intensity and polarization of intraspecific aggression at the worker level, we used standardized behavioral assays (Carlin and Hölldobler, 1986; Holway et al., 1998; Roulston et al., 2003; Suarez et al., 1999, 2002; Tsutsui et al., 2003). We quantified the level of intraspecific aggression between subcolony pairs at two time intervals for all supercolony pairings: before subcolonies were connected and at 2 days after connection. Two additional time intervals, 6 weeks and 12 weeks after connection, were tested for the large supercolony versus small supercolony pairings.

For each trial, we haphazardly selected a single worker from each nesting container within a pair and placed the two workers together in a plastic vial with Fluon™-coated sides for 1 h. By feeding one of the ants a small amount of dilute sugar water, we were able to distinguish workers from the two subcolonies during the trials by differences in abdomen size (Tsutsui et al., 2003). In successive trials, we alternated the colony identity of marked (fed) individuals. All trials were done blind. We scored the behavioral interactions that ensued in order of escalating aggression: ignore = 0 (contacts between individuals where neither ant showed any interest in the other), touch = 1 (contacts that included prolonged antennation), avoid = 2 (contacts that resulted in one or both ants retreating in opposite directions), aggression = 3 (lunging, biting, and pulling legs or antennae), or fight = 4 (prolonged aggression between individuals). For each trial, we recorded the highest level of aggression observed. If this level was 3 or 4, we also recorded which ant initiated the fight.

For each subcolony pairing, we performed 10 behavioral assays for each time interval. We also conducted behavioral assays between workers from unconnected control subcolonies to account for changes in aggressiveness that may have occurred due to laboratory conditions. No worker was used in more than one bioassay.

To quantify whether asymmetrical aggression shifted to symmetrical aggression after colonies made contact with each other, we calculated an index of polarity for each pairing of supercolonies. To calculate this index, we first determined the mean proportion of trials for which workers from one subcolony initiated aggression against workers from the other subcolony. The index of polarity is the absolute difference between these two proportions and reflects the extent to which polarized aggression occurs for each subcolony pairing. An index equal to 1 would indicate that workers from one subcolony always initiated aggression against workers from the other subcolony, whereas an index equal to 0 would indicate that workers from the two subcolonies initiated aggression against one another with equal frequency. We calculated the mean polarity index within each supercolony pairing and used separate paired *t* tests to compare (1) control versus experimental supercolony pairs prior to connection of subcolonies in the experimental treatment and (2) control versus experimental supercolony pairs after connection of subcolonies in the experimental treatment.

Field experiments

Border transects: naive versus experienced workers

To test the relationship between intraspecific aggression and behavioral asymmetries in the field, we compared the behavior of workers from established nests at supercolony boundaries (experienced workers) with the behavior of workers from nests located 500 m away from colony boundaries (naive workers). We sampled workers from nine transects that traversed the large supercolony and LH_{S1} borders. All border locations were at least 1 km apart. It was not possible to do this experiment using workers from the large supercolony and LS_{S2} because these two supercolonies do not currently contact each other. Similarly, we did not conduct bioassays between the large supercolony and SW_{S3} supercolonies as there are only two known locations where these supercolonies physically meet.

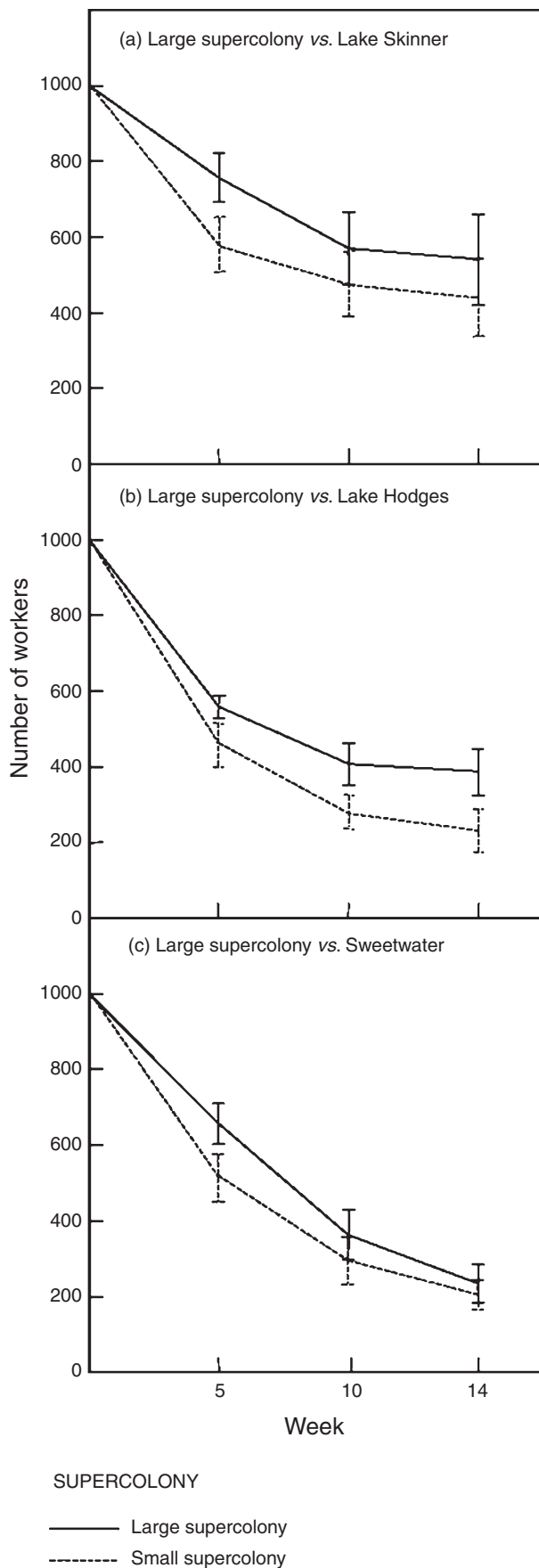
Behavioral assays were undertaken in the laboratory as described in the Worker-Level Aggression—All Supercolony Pairings. Ten bioassays were performed per transect per treatment (naive or experienced). We used paired *t* tests (border versus 500 m from border, paired per transect) to test for differences between naive and experienced workers in the level of aggression, the time until aggression escalated to a level 3 or 4, and the symmetry of aggression. We used the polarity index (see above) as the dependent variable, and each border site was considered a replicate.

Introduction experiments

The colony-level laboratory experiments described above allow great control over colony size, available resources, and physical conditions but potentially provide a misleading picture of competitive relationships, given that ant colonies are confined to a structurally simple and confined space. To gain additional insights into colony-level disparities in competitive performance, we also conducted short-term introduction experiments in the field. These introductions involved the release of an experimental Argentine ant colony of controlled size in the vicinity of a recruitment trail of an established field colony.

Bioassays between supercolonies were as follows, LP_L (representing the large supercolony)—LH_{S1}, LP_L—LS_{S2}, and LP_L—SW_{S3}. These bioassays were done reciprocally. For example, a colony fragment collected from LP_L was introduced onto a foraging trail at LH_{S1}, and an LH_{S1} colony fragment was introduced onto a foraging trail at LP_L. As in the colony-level laboratory experiment, we did not test smaller supercolonies against each other. Bioassays were conducted at 10 locations that were at least 200-m distant from each other at LP_L, LH_{S1}, and SW_{S3}. Due to the relatively small size of the LS_{S2} supercolony, bioassays at this site were carried out at six locations (at least 10 m apart) spread throughout the territory of this supercolony. At each bioassay location, we also conducted bioassays using ants from the same supercolony as a control.

The colony fragments that were introduced onto field colony foraging trails were collected from 10 different locations at LP_L, LH_{S1}, and SW_{S3}. The distance between collection sites within supercolonies was at least 200 m. Due to the small size of LS_{S2} and the difficulty in obtaining nests from this site, we collected ants at only one location within this supercolony. Collected colony fragments were separated into groups consisting of two queens and approximately 1000 workers and 100 pieces of brood, with a total of 92 subcolonies (2 from each site of collection at LH_{S1} and SW_{S3}, 4 from each LP_L site, and 12 from the one LS_{S2} collection site). These subcolonies were housed in round plastic containers (20 cm in diameter) lined with Fluon™. We provided ants with nesting chambers consisting of test tubes covered with aluminum foil, half filled with water,



and plugged with cotton wool. We fed subcolonies sugar water daily and crickets twice per week and kept them at 25°C until used in field bioassays. The morning prior to each bioassay, nest containers were fitted with a plugged 30-cm exit tube.

To quantify resource consumption, we modified an approach developed by Morrison (1999). Bioassays were performed 30 cm from the entrance to a nest in the field. A 1-g piece of tuna in oil was placed on an index card (7.5 by 12.5 cm) on the foraging trail leading from the nest entrance, and workers were allowed to recruit to the tuna for 15 min. The distal end of the laboratory subcolony's exit tube was positioned on the opposite edge of the index card to the focal bait, 5 cm from the bait. If after 15 min, the number of ants feeding on the bait was between 10 and 16 (mean = 12.71 ± 2.46 workers), we unplugged the exit tube of the laboratory subcolony, permitting the free passage of workers from the nest container to the area surrounding the tuna. Then, every 5 min, we recorded the number of ants feeding at the tuna. We also recorded the number of fights initiated on the index card and the number of ants exiting and entering the introduced subcolony's tube. After 50 min, we measured resource consumption by reweighing the tuna. To control for loss of weight through evaporation, five additional 1-g pieces of tuna were also weighed, placed near the focal bait in Fluon™-coated petri dishes that excluded ants, and then reweighed at the conclusion of the trial.

Resource consumption was calculated as the weight loss of each piece of tuna fed on by ants minus the weight lost from evaporation (estimated as the mean weight loss of five pieces of tuna not fed on by ants). We used paired *t* tests to analyze differences in resource consumption of the field colonies when an aggressive subcolony was released onto the foraging trail (experiment) or when a subcolony from the same supercolony was introduced (control). Because resource consumption is confounded by differences in recruitment levels between supercolony sites (see Results), we have used resource consumption per worker to test for differences in resource consumption between large and small supercolonies. Bioassays were conducted blind such that the order of introductions at each site was conducted at random. All data were analyzed using the software package SYSTAT 9.0 (Wilkinson, 1998). All errors are standard errors if not otherwise stated.

RESULTS

Laboratory experiments

Colony-level competition—large supercolony versus small supercolony pairs

After 14 weeks, there was no significant difference in worker number between the small supercolonies and the large supercolony (LH_{S1}: $t = 1.85$, $df = 4$, $p = .14$; LS_{S2}: $t = 0.55$, $df = 5$, $p = .61$; SW_{S3}: $t = -0.80$, $df = 5$, $p = .46$). This pattern was observed at all time points throughout the observation period (Figure 1). Similarly, brood production did not differ between the large and small supercolonies at 14 weeks (LH_{S1}: $t = 0.60$, $df = 4$, $p = .58$; LS_{S2}: $t = 0.34$, $df = 5$, $p = .75$;

←

Figure 1

Results of colony-level laboratory experiments showing mean (±1 SE) numbers of workers surviving at 0, 5, 10, and 14 weeks for (a) large supercolony versus Lake Skinner colony pairings, (b) large supercolony versus Lake Hodges, and (c) large supercolony versus Sweetwater. "Large" and "small" refer to the colony size in the field; experimental colonies were of equal size. There was no significant difference in worker number at any time interval between the small supercolony and the large supercolony.

SW_{S3}: $t = 1.36$, $df = 5$, $p = .23$). Overall, worker mortality was high over the course of the experiment, with only 20–60% of workers surviving for the entire 14-week period (Figure 1). We never observed colony pairs fusing.

Foraging activity was observed for a total of 46 days. During this time, the number of days a given colony was observed foraging did not differ between subcolonies in each supercolony pairing (paired t test—LH_{S1} colony pairs: LH_{S1} = 18.00 ± 6.73 days, large supercolony = 18.20 ± 6.15 days, $t = 0.02$, $df = 4$, $p = .99$; LS_{S2} colony pairs: LS_{S2} = 9.33 ± 6.62 days, large supercolony = 25.16 ± 6.95 days, $t = 1.23$, $df = 5$, $p = .27$; SW_{S3} colony pairs: SW_{S3} = 18.50 ± 4.90 days, large supercolony = 16.00 ± 6.04 days, $t = -0.23$, $df = 5$, $p = .83$). On a few occasions, workers from both subcolonies were seen in the foraging arena at the same time (LH_{S1} pairings = 5.7% of days, LS_{S2} = 2.9%, SW_{S3} = 5.1%), but it was rare for there to be no foraging activity (LH_{S1} = 3.0% of days, LS_{S2} = 7.6%, SW_{S3} = 4.0%).

The number of days that each subcolony was observed retrieving flies did not differ between the two subcolonies in each pairing (paired t test—LH_{S1} colony pairs: LH_{S1} = 6.20 ± 1.66 days, large supercolony = 5.80 ± 1.69 days, $t = 0.12$, $df = 4$, $p = .91$; LS_{S2} colony pairs: LS_{S2} = 3.17 ± 2.23 days, large supercolony = 9.17 ± 2.14 days, $t = -1.40$, $df = 5$, $p = .22$; SW_{S3} colony pairs: SW_{S3} = 6.50 ± 2.23 days, large supercolony = 5.83 ± 2.33 days, $t = 0.15$, $df = 5$, $p = .89$). On days when flies were retrieved, all six *Drosophila* were always retrieved by the same subcolony.

Worker-level aggression—all supercolony pairings

The level of aggression between pairs of experimental subcolonies was significantly higher 2 days after subcolonies had been connected than it was prior to connection (aggression rating before connection = 2.30 ± 0.21 , after connection = 3.28 ± 0.13 ; paired t test: $t = 5.06$, $df = 5$, $p < .001$). There was no significant change in the level of aggression between unconnected (control) subcolonies (before = 2.40 ± 0.26 , after = 2.30 ± 0.30 ; paired t test: $t = -0.53$, $df = 5$, $p = .61$). The aggression level between the large supercolony and small supercolony pairings remained high for the entire 12-week period after colony connection (Figure 2).

Patterns of asymmetric aggression also changed during the course of the experiment. Initially, aggression was asymmetrical in five of the six supercolony pairings, with workers from both the large supercolony and SW_{S3} more likely to initiate aggression than workers from LH_{S1} or LS_{S2} (polarity index: control = 0.78 ± 0.02 , experiment = 0.67 ± 0.02 ; paired t test: $t = 1.42$, $df = 4$, $p = .23$) (Figure 3a). However, after subcolonies were connected, aggression between colonies became symmetrical in all five cases (polarity index: control = 0.78 ± 0.03 , experiment = 0.03 ± 0.001 ; paired t test: $t = 11.74$, $df = 4$, $p < .001$) (Figure 3b). This shift from asymmetrical, polarized aggression to symmetrical, unpolarized aggression occurred only among connected, experimental subcolonies; it did not occur among unconnected, control subcolonies (Figure 3a,b). In addition, the observed change in aggression symmetry was consistent for the entire 12 weeks in the large supercolony versus small supercolony pairings. In one experimental pairing (large supercolony versus SW_{S3}), aggression was not asymmetrical either at the beginning of the experiment or after 12 weeks. Workers from these subcolony pairings were equally likely to initiate aggression at each time point.

Field experiments

Border transects: naive versus experienced workers

There was a difference in the symmetry of aggression between experienced and naive workers (polarity index at border =

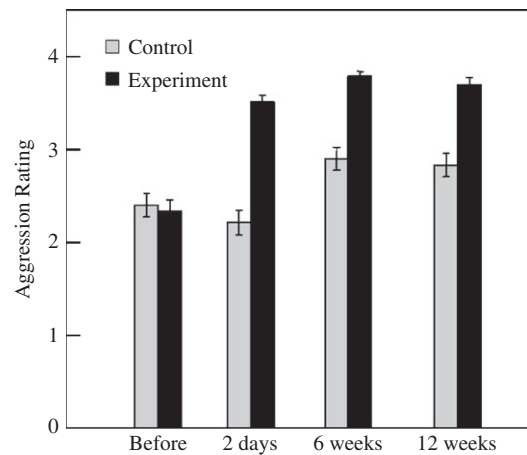


Figure 2

Results of worker-level aggression trials showing the mean (± 1 SE) level of aggression exhibited by worker pairs before colonies were connected and 2 days, 6 weeks, and 12 weeks after connection of experimental colonies. Only the large supercolony versus small supercolony worker pairs are included. Black bars represent experimental treatments where aggressive colonies were connected to each other, and gray bars represent colonies that remained unconnected for the duration of the experiment (control colonies). The level of aggression between pairs of experimental subcolonies was significantly higher after subcolonies had been connected than it was prior to connection; aggression remained high for the duration of the experiment (12 weeks). Aggression between control subcolony pairs did not change after subcolonies were connected.

0.13 ± 0.14 , 500 m from border = -0.38 ± 0.13 ; paired t test: $t = 4.08$, $df = 8$, $p = .004$). In the naive pairings, workers from the large supercolony were more likely to initiate aggression. However, in the border pairings, both supercolonies were equally likely to initiate aggression. This difference in asymmetry also coincided with differences in the intensity of aggression and in the time it took pairs of workers to start fighting. Worker pairings from the border escalated to aggression faster (mean time until aggression: border = 162.49 ± 40.90 s, 500 m from border = 367.82 ± 49.20 s; paired t test: $t = -3.92$, $df = 8$, $p = .004$) and were more aggressive toward each other (mean aggression rating: border = 3.73 ± 0.09 , 500 m from border = 2.84 ± 0.15 ; paired t test: $t = 5.18$, $df = 8$, $p = .001$) than workers taken 500 m from the border.

Introduction experiments

In aggressive pairings, large and small supercolonies retrieved similar amounts of tuna per capita (Table 1, Figure 4). Performing the same analyses on absolute measures of resource exploitation yields the same qualitative pattern.

Although large and small supercolonies did not differ in terms of resource retrieval, five times more tuna was consumed when subcolonies from the same supercolony were introduced onto the foraging trail (control) than when a subcolony from a different supercolony was introduced (experiment) (Table 2). Compared to experimental trials, control trials had significantly more workers feeding at the tuna bait, and fights occurred rarely (Table 2). The activity of the laboratory subcolonies (measured as the number of ants exiting the laboratory colony minus the number of workers entering) was also significantly higher in the control than in treatment bioassays (Table 2).

Resource consumption was positively related to the number of ants that recruited to baits ($r^2 = .42$, $n = 91$, $p < .001$). However, the overall number of ants feeding at baits differed

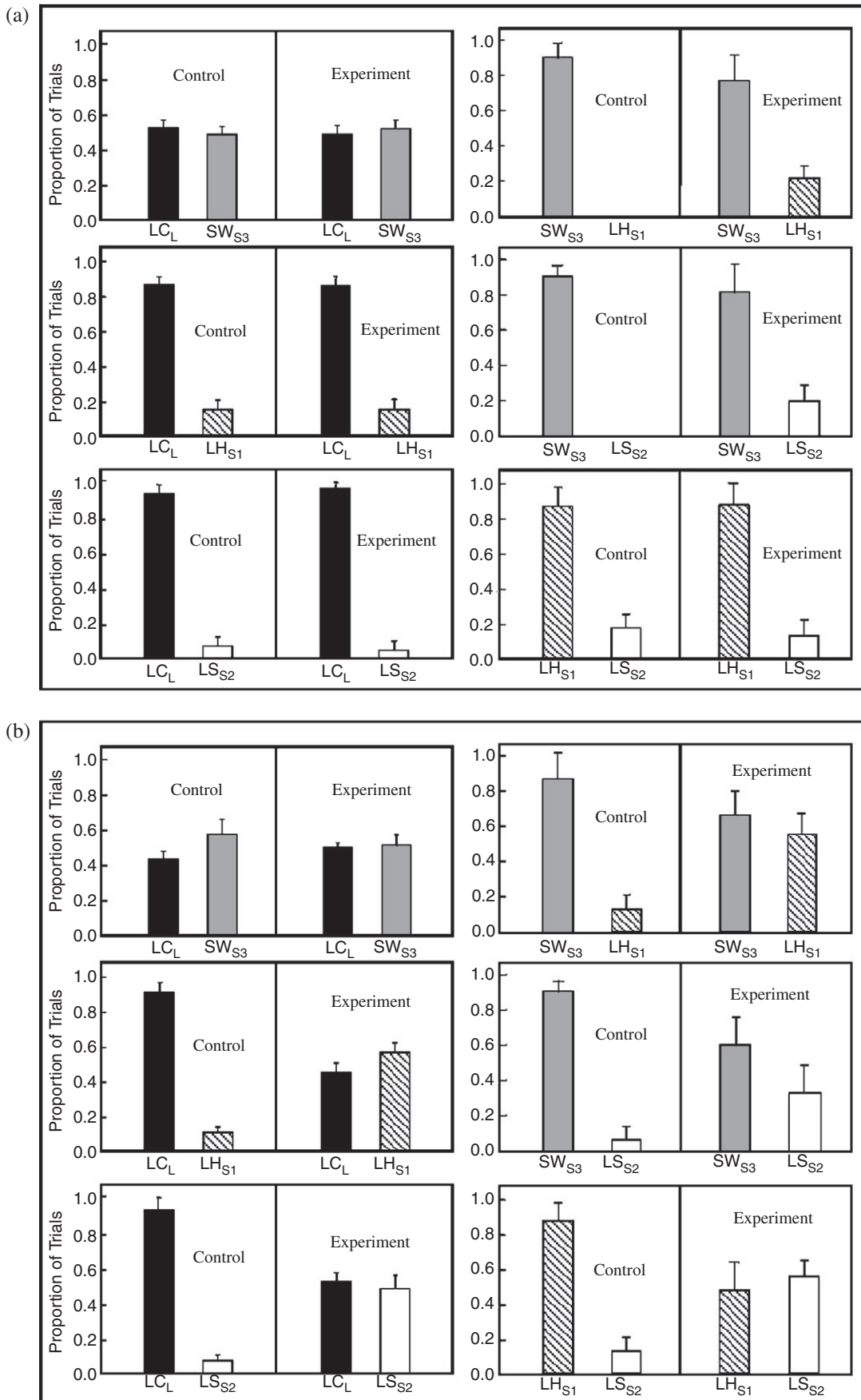


Table 1

Table lists mean per capita resource consumption (mg/worker) for control and experimental trials grouped by small supercolony

Colony pair	Treatment	Mean LC _L (mg/worker)	Mean SC _S (mg/worker)	<i>t</i> value	df	<i>p</i>
Large supercolony versus Lake Hodges	Control	0.92	0.96	0.039	9	.970
	Experiment	-1.35	-0.63	0.432	9	.676
Large supercolony versus Lake Skinner	Control	1.26	3.46	-0.955	4	.394
	Experiment	13.91	3.51	-1.179	6	.283
Large supercolony versus Sweetwater	Control	0.92	1.50	-0.573	9	.581
	Experiment	-0.52	7.11	2.716	9	.024

LC_L = Large supercolony and SC_S = small supercolonies. Bonferroni adjusted *p* value for six multiple comparisons is .008.

significantly among locations, with the highest numbers at LH_{S1} and LS_{S2} (LH_{S1}: 54.37 ± 11.80 ants, *n* = 10; LS_{S2}: 43.44 ± 10.29, *n* = 5; SW_{S3}: 17.64 ± 2.78, *n* = 10; LP_L: 16.82 ± 2.67, *n* = 10; ANOVA: $F_{3,32} = 6.76$, *p* = .001 [only control trials included]).

DISCUSSION

Previous studies have documented that intraspecific aggression can be asymmetrical between workers from different Argentine ant colonies (Tsutsui et al., 2003). The pattern of polarized aggression observed at the beginning of our laboratory experiment and between naive workers from the border transects exactly matches that reported by Tsutsui et al. (2003). However, we found that this asymmetry always disappears when workers have experience with a foreign, conspecific colony (Figure 3). This unexpected finding may help explain why we obtained little evidence for asymmetries in colony-level performance or productivity (Figures 1 and 4) in both the laboratory and field colony-level experiments. Moreover, this result provides insights into both the ontogeny of recognition systems in ants and the costs (or lack thereof) associated with asymmetrical aggression between Argentine ant colonies.

The observed shift from asymmetrical to symmetrical aggression agrees broadly with previous work showing that social insects can modify their aggressive behavior based on prior experience with individuals from foreign colonies. In some studies, individuals from neighboring colonies displayed lower levels of aggression toward each other than toward individuals from more distant colonies (the dear enemy phenomenon; Jutsum et al., 1979; Langen et al., 2000; Thomas et al., 1999), whereas other studies have reported the opposite pattern, with individuals from neighboring colonies displaying a higher level of aggression toward each other than to more distant colonies (Dunn and Messier, 1999; Gordon, 1989; Sanada-Morimura et al., 2003). In our study, workers displayed heightened aggression toward previously encoun-

tered colonies, suggesting the opposite of the dear enemy phenomenon. This behavioral shift most likely resulted from direct interactions among individuals from different subcolonies because this transition did not occur in unconnected, control subcolonies (Figure 2) or among workers collected 500 m from supercolony borders.

The shift from asymmetrical to symmetrical aggression coincided with both an increase in the number of aggressive encounters and an increase in the level of aggression displayed by fighting workers in both the laboratory and the field experiments. In the large supercolony versus SW_{S3} pairing, where aggression increased but symmetry did not appear to change (Figure 3), individual trials achieved higher aggression scores without an increase in the number of aggressive encounters. The higher aggression scores observed among colonies that had experience interacting with one another could reflect differences in perceived costs and benefits associated with the changed ecological environment before and after colony connection. Prior to colony connection, the one-on-one bioassays closely resembled isolated encounters between foragers away from their nest. However, after colony connection, workers were constantly defending their nest and sole food source from a foreign aggressive colony. These observations are generally consistent with previous work showing that workers exhibit high levels of aggression when competing for food (e.g., Cerdá et al., 1998; Fellers, 1987; Holway, 1999) or when defending their nests from foreign ants (e.g., Hölldobler, 1976). The heightened aggression observed in the individual fighting trials after colony connection was not an artifact of culture under laboratory conditions because workers from control colonies did not show a similar increase in aggression.

Although equal (and low) levels of genetic diversity in both SW_{S3} and the large supercolony may give rise to the symmetry in aggression between these two colonies (Tsutsui et al., 2003), an alternative explanation could be the presence of a supercolony boundary near the collection site for SW_{S3}. In this experiment, and in both Suarez et al. (2002) and Tsutsui

Figure 3

Results of worker-level aggression trials showing the mean (±1 SE) proportion of times workers from each supercolony were the aggressors in one-on-one conflicts (a) before colonies were connected and (b) 2 days after connection of experimental colonies. Only aggressive encounters that rated a 3 or 4 on the aggression index are included (see Methods). Aggressive colonies were connected to each other in experimental treatments and remained unconnected for the duration of the experiment in control treatments. Black bars = large supercolony (LC_L), gray bars = Sweetwater (SW_{S3}), hatched bars = Lake Hodges (LH_{S1}), and white bars = Lake Skinner (LS_{S2}). Prior to colony connection, workers from the large supercolony and SW_{S3} were typically the attackers when paired with ants from either Lake Hodges or Lake Skinner. After colony connection, aggression became symmetrical in the experimental treatments.

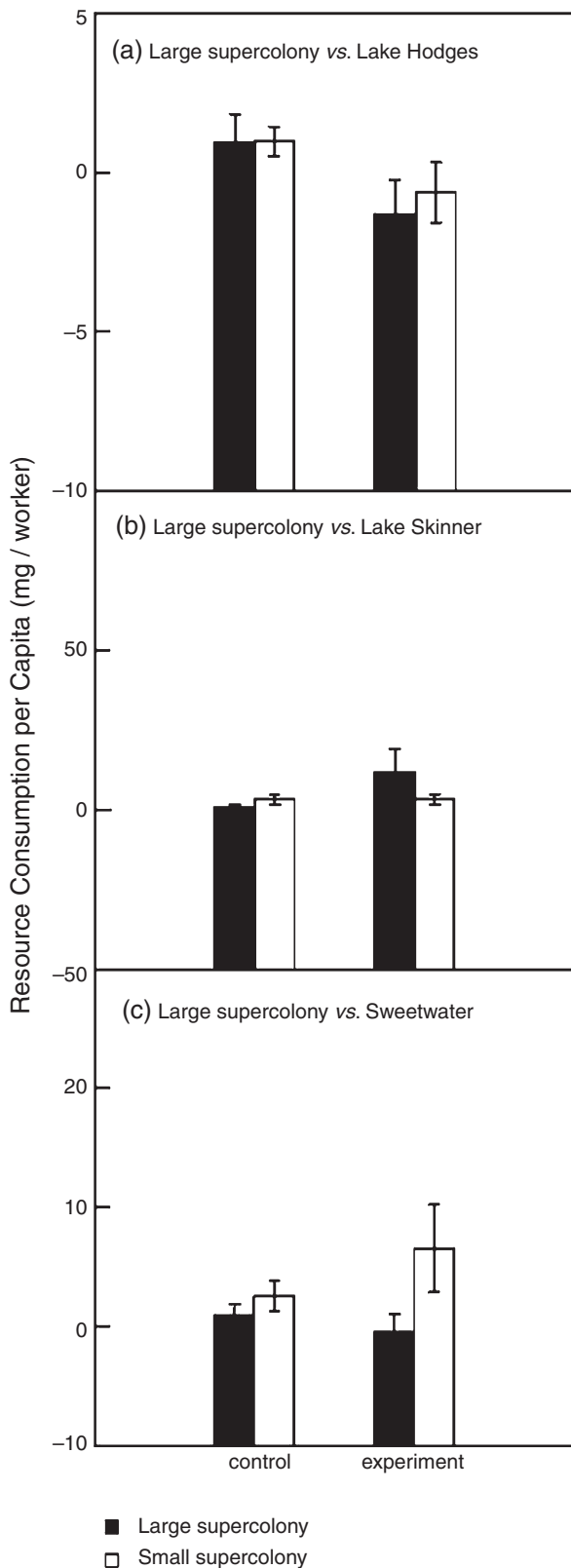


Figure 4
Results of the field experiment showing mean (± 1 SE) per capita resource consumption (mg/worker) for (a) large supercolony versus Lake Hodges colony pairings, (b) large supercolony versus Lake Skinner, and (c) large supercolony versus Sweetwater. Each bar represents the amount of resource consumed by the resident field colony when a control nest (subcolony from the same supercolony) or

Table 2

Table lists mean (± 1 SE) values for four different measures of competitive performance and results of *t* tests comparing control ($n = 36$) and experimental ($n = 55$) trials

	Control	Experiment	<i>t</i>	df	<i>p</i>
Resource consumption (g)	0.052 \pm 0.068	0.011 \pm 0.041	3.569	90	.001
Number of workers feeding at bait (workers/min)	6.24 \pm 5.49	2.13 \pm 2.30	4.985	90	<.001
Activity of laboratory colony (workers/min)	5.36 \pm 1.79	1.44 \pm 1.14	12.821	90	<.001
Number of fights/min	0.023 \pm 0.13	0.620 \pm 0.282	-11.909	90	<.001

Control trials involved bioassays conducted within the same supercolony; experimental trials involved bioassays conducted between different supercolonies. Significant *p* values are in bold.

et al. (2003), colony fragments of SW_{S3} were collected within the vicinity of a contact zone between SW_{S3} and the large supercolony. If prior experience is an important determinant of aggression between colonies, as our results suggest, this may account for a lack of polarity observed between these supercolony pairings both in our study and in Tsutsui et al. (2003). Unfortunately, due to an insufficient number of border sites ($n = 2$), we were unable to determine if this scenario is correct.

There are at least two alternative explanations that could account for the observed shift from asymmetric to symmetric aggression in the laboratory experiment. First, the high levels of worker mortality in our laboratory colonies could have altered the costs or benefits of aggressive behavior so as to favor increasing hostility with decreasing colony size. Preliminary field studies also indicate a high level of worker mortality at supercolony borders. Future studies that examine the relationship between colony size and worker recognition behavior would clarify this issue. Second, the high level of asymmetric aggression that initially occurred in five of the subcolony pairs may have eliminated many of the non-aggressive workers in the colony that was typically the recipient of aggression, leaving only workers that display high levels of aggression toward the attacking colony workers. Previous work has shown that, subsequent to hostile interactions, attacking workers experience a much higher survival rate compared to workers that are the recipients of aggression (Tsutsui et al., 2003), although we did not observe a significantly higher level of worker mortality in colonies that were the recipients of aggression. Nevertheless, future experiments that track behavioral changes of individual workers will permit a direct test of these possibilities.

The behavioral plasticity we report here sheds light on the origin of uniclonality in invasive ants. In some cases, a decrease in intercolony aggression has been proposed as a mechanism by which initially hostile, multicolonial populations could coalesce into widely cooperative supercolonies (Giraud

←

experimental nest (subcolony from an intraspecifically aggressive supercolony) was introduced. Filled bars for the large supercolony and empty bars for the small supercolonies represent the resource consumption per worker for the resident field colonies. Pairings do not differ significantly from one another (see also Table 2).

et al., 2002; Nonacs, 1993; Ross and Keller, 1995; Ross et al., 1996). Our data, however, suggest that encounters between initially hostile colonies act to increase levels of aggression between them. In addition, we saw no evidence of colony fusion between laboratory subcolonies during the 14-week experimental period. Long-term observations of naturally occurring territorial boundaries are needed to test these ideas in more detail.

Other studies have suggested that asymmetries in genetic diversity underlie the observed asymmetries in aggression between Argentine ant colonies (Tsutsui et al., 2003). Thus, when there is a fitness cost associated with being attacked (e.g., differential mortality between attackers and recipients), low-diversity colonies may be at a selective advantage relative to high-diversity colonies, leading to a continued loss of genetic diversity in introduced populations (Tsutsui et al., 2003). Although our results are consistent with a genetic basis for asymmetrical aggression, they also suggest that workers from high-diversity colonies are quickly able to learn an opposing colony's odor and react in an aggressive manner. Therefore, although asymmetrical aggression should occur when different colonies encounter each other for the first time, the selective pressure on high-diversity colonies produced by asymmetrical aggression should rapidly disappear as aggression becomes more symmetrical.

CONCLUSIONS

Our results help to illustrate the dynamic nature of social insect recognition systems, with the ontogeny of nest-mate recognition systems emerging as a multistage process. Imprinting soon after eclosion allows individuals to define a set of cues (or labels) that are acceptable. Later in life, experience-based learning may act to more finely tune recognition, as individuals become sensitized to (and hence, behave more aggressively toward) individuals from competing colonies. Our data clearly show that social insect recognition systems can develop as individuals acquire novel, experiential information and integrate this information into the preexisting recognition system. This process of defining "self" during an early critical period, then later refining the definition of "nonself" based on experience may be a common feature of recognition systems generally.

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