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# Aggressive interactions between the introduced Argentine ant, *Linepithema humile* and the native odorous house ant, *Tapinoma sessile*

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**Abstract** The Argentine ant (*Linepithema humile*) is an invasive species that disrupts the balance of natural ecosystems by displacing indigenous ant species throughout its introduced range. The mechanisms by which Argentine ants effectively compete against native ant species have been previously addressed in field studies that centered on interference and exploitation competition at baits and mainly examined the colony-level performance of Argentine ants. Detailed behavioral observations explaining the basis for the strong competitive ability of *L. humile* are comparatively rare. To gain a better understanding of the mechanisms by which Argentine ants displace native ants we examined the aggressive interactions between the Argentine ants and the odorous house ant, *Tapinoma sessile* in four different aggression assays: (1) worker dyad interactions, (2) symmetrical group interactions, (3) intruder introductions into an established resident colony, and (4) a resource competition assay which focused on competition for food and nesting space. Our results demonstrate a clear disparity between worker-level and colony-level fighting ability of Argentine ants and provide behavioral evidence to explain the superior interference ability of Argentine ants in group assays. Argentine ants experienced mixed success in fighting against odorous house ants in dyad interactions, but gradually gained a numerical advantage in symmetrical group interactions by active cooperation among nestmates. Results of the resource competition assay indicate that Argentine ants recruit rapidly, numerically dominate food and nesting sites, and aggressively displace *T. sessile* from baits. Taken together, the results of these assays allow us to pinpoint the behavioral mechanisms responsible for the remarkable competitive ability of Argentine ants.

**Keywords** Aggression assay · Argentine ant · Competition · Invasive ants · *Linepithema humile* · Odorous house ant · *Tapinoma sessile*

## Introduction

Invasive ants are a key threat to natural ecosystems and agriculture making it essential to understand the factors responsible for their remarkable ability to compete in newly invaded environments. The Argentine ant, *Linepithema humile* (Mayr), is an introduced and invasive species that has spread nearly worldwide (Suarez et al. 2001; Roura-Pascual et al. 2004). Within introduced populations, *L. humile* exhibits a unicolonial colony structure consisting of large, multiple queen colonies that lack clear boundaries due to a general absence of intraspecific aggression (Suarez et al. 1999; Tsutsui et al. 2000; Giraud et al. 2002). When invading novel environments, Argentine

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ants face two enormous tasks. First, they must adapt to their new habitat and often form intricate mutualistic relationships with honeydew-producing Homoptera, with which they share no previous evolutionary history. Second, they must contend with an array of native ant species and other arthropods that are already adapted locally with which they may directly or indirectly compete for food and nesting space. Despite these potential obstacles, the invasion of Argentine ants is associated with the loss or reduced abundance of native ant species throughout its introduced range (Holway 1998, 1999; Holway et al. 2002; Human and Gordon 1999; Touyama et al. 2003). The interactions between the Argentine ant and native ants have been examined in numerous studies (e.g., Human and Gordon 1996; Holway 1998, 1999; Thomas and Holway 2005; Zee and Holway 2006; Rowles and O'Dowd 2007) that indicate the competitive ability of Argentine ants stems from numerical dominance (i.e., formation of large supercolonies; Holway and Suarez 2004), aggressiveness (Human and Gordon 1999), superior interference and exploitation competition (Human and Gordon 1996; Holway 1999), and ability to quickly recruit to food (Holway 1999). The majority of studies to date have been field experiments that centered on interspecific competition at baits and mainly examined the colony-level performance of Argentine ants. However, detailed laboratory examinations that test worker-level performance and the behavioral mechanisms responsible for the invasive spread of *L. humile* are lacking.

In this study, we examined the aggressive interactions between the introduced Argentine ant, *L. humile* (Mayr) and the native odorous house ant, *Tapinoma sessile* (Say). We chose *T. sessile* as an interspecific competitor because its biology, morphology, behavior, and colony attributes are remarkably similar to those of *L. humile*. Such similarities may be an important advantage when evaluating interactions between the two species because the effect of extraneous factors such as worker size, dietary requirements, and colony attributes are minimized. Both *L. humile* and *T. sessile* belong to the subfamily Dolichoderinae (Shattuck 1992) and both have monomorphic workers with similar size and behaviors (quick moving, able to use physical and chemical defenses). Both species form large polygynous and polydomous colonies with tens of thousands of workers (Markin 1970; Buczkowski and Bennett 2006). Both are associated with anthropogenically disturbed habitats (Passera 1994; Buczkowski and Bennett 2006) and are opportunistic, inhabiting a variety of natural and man-made nesting sites. Both build impermanent nests that are frequently moved and both are considered pests (Markin 1970; Thompson 1990). Both species also share numerous similarities in their foraging ecology including: mass-recruitment foraging strategy along well defined trails, similar daily activity patterns, and similar dietary preferences with heavy reliance on homopteran excretions. Within the United States, the Argentine ant is found mainly in California (Holway 1995) and several southeastern states (Buczkowski et al. 2004). The odorous house ant is a native species widely distributed throughout North America (Creighton 1950) and its range widely overlaps with that of the Argentine ant (Holway 1999; Human and Gordon 1999).

We examined interactions between *L. humile* and *T. sessile* in four different aggression assays: (1) worker dyad interactions, (2) symmetrical group interactions, (3) intruder introductions into an established resident colony, and (4) a resource competition assay which focused on competition for food and nesting space. Worker dyad interactions tested the performance of individual Argentine and odorous house ants in the absence of any numerical advantages. Symmetrical group interactions examined the competitive ability of the two species in group fights where nestmates could cooperate in fighting against the opposing species and numerical advantages could develop as one species dominated over the other. Intruder introductions tested the defensive ability of whole colonies against individual workers and vice versa. The resource competition assay examined the aggressive interactions between the two species in a large arena assay that focused on competition for food and nesting space. Taken together, the results of these assays allow us to pinpoint the behavioral mechanisms responsible for the remarkable competitive ability of Argentine ants.

## Materials and methods

### Collection and maintenance of laboratory colonies

Argentine ants, *L. humile* (Mayr) were collected on the campus of Genentech Inc., in South San Francisco, California (SSF) and in Research Triangle Park, North Carolina (RTP). Odorous house ants, *T. sessile* (Say) were collected on the campus of Purdue University in West Lafayette, Indiana (PUR), and the Entomology Field Operations Building, West Lafayette, Indiana (EFO). These collections encompass colonies representing various geographic regions and population types. SSF represents Argentine ants that most likely belong to the large supercolony previously described from California (Suarez et al. 1999; Tsutsui et al. 2000). RTP represents Argentine ants from the southeastern United States, where Argentine ants are behaviorally and genetically distinct from those in the California supercolony (Buczkowski et al. 2004). PUR represents odorous house ants from a large supercolony comprised of numerous nests (Buczkowski and Bennett 2006) and EFO represents a smaller, polydomous colony. For each species, we established a single colony consisting of 5,000–10,000 workers, a few hundred queens, and numerous brood. Given the unicolonial nature of *L. humile* and *T. sessile*, we collected ants from numerous nests, but raised them as a single colony. Colonies of both species were maintained in soil-free, Fluon$^{TM}$-coated trays containing moist plaster nests. Both species were provisioned with 20% sucrose solution and artificial diet (Bhatkar and Whitcomb 1970) ad libidum, and hard-boiled egg once a week. All colonies were maintained at 24 ± 1°C, 50 ± 10% RH, and a 12:12 L : D cycle.

## Aggression tests

We assessed the level of aggression between four colony pairs: SSF–PUR, SSF–EFO, RTP–PUR, and RTP–EFO using four different aggression bioassays: worker dyad interactions within a neutral arena, group worker interactions in a neutral arena, intruder introductions into an established resident territory and a large arena assay that focused on competition for food and nesting space. Detailed descriptions of each assay are presented below. All aggression assays were conducted blind: the observer who recorded worker aggression levels did not know the source of the interacting ants and was unfamiliar with the hypothesis being tested. In all assays, individual ants were not tested in more than one trial.

### Assay 1: dyad interactions

The objective for this one-on-one assay was to determine the fighting ability of individual Argentine and odorous house ant workers. Argentine and odorous house ant workers, selected at random from stock colonies, were collected on a toothpick and placed sequentially into a glass vial (two-dram). The top half of each vial was coated with Fluon$^{TM}$ to restrict the ants to a small area to maximize the chance of the ants finding each other. Ant interactions were scored on a 1–4 scale (Suarez et al. 2002) [1 = ignore, 2 = avoid, 3 = aggression (lunging, brief bouts of biting, and/or pulling), 4 = fighting (prolonged aggression, also abdomen curling to deposit defensive compounds)]. We examined the level of aggression between Argentine and odorous house ants using four colony pairs: SSF–PUR, RTP–PUR, SSF–EFO, and RTP–EFO and performed ten replicates for each pair. In each replicate we allowed the ants up to 25 encounters, each instance of direct physical contact between the ants was regarded as an encounter. For each replicate we recorded: the maximum score of 25 encounters (Roulston et al. 2003), the fight initiator, the fight winner, the number of interactions for the winner to kill the opposing ant, and the mechanisms employed by both species (i.e., physical aggression, chemical defenses, or both).

### Assay 2: symmetrical group interactions in a neutral arena

This assay was designed to test the competitive ability of the two species in group fights utilizing an equal starting number of workers for each species. As the fights progressed, one species could gain a numerical advantage over the other by having the uninjured survivors of fights join their nestmates in fighting the opposing species. We followed the protocol of Buczkowski and Silverman (2005). Twenty randomly selected workers were transferred to a plastic, Fluon$^{TM}$-coated dish (9 cm diameter and 2 cm high). Similarly, 20 workers were placed in a plastic Fluon$^{TM}$-coated arena ($30 \times 17 \times 8$ cm$^3$ 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 dish into the arena. The same four colony pairs were tested and we performed three replicates for each pairing. The number of ants involved in fighting (aggression level 3 or above) was recorded at 1, 5, 10, 20, 40, and 60 min after the two species were mixed and then every hour until 8 h. At each time point we also recorded for each species: the number dead and the ratio of workers of each species involved in fights.

### Assay 3: intruder introduction to resident territory

The objective for this assay was to examine the defensive ability of a resident colony toward individual intruding workers of the other species. Such numerical asymmetries may be common during colonization events or at invasion fronts, where one species may have a substantial numerical advantage over the other. A secondary goal was to test our initial observation that Argentine ant workers cooperate in killing other ants, whereas odorous house ants tend to fight alone and do not usually receive any help from their nestmates. Individual intruder workers were collected on a toothpick and introduced into rearing trays ($52 \times 38$ cm$^2$) containing a resident colony (10,000 workers). Aggression was scored using the 1–4 scale of Suarez et al. (2002). For each test, we allowed the intruder up to 25 encounters with resident ants. The intruder was discarded after each trial, and subsequent trials were conducted when the residents were no longer visibly agitated (5–10 min). The same four colony pairs were tested and we performed ten replicates for each pairing. Thus, we performed 40 replications with Argentine ant workers acting as intruders, and 40 replications with odorous house ant workers acting as intruders. For each replicate we recorded: the maximum score of 25 encounters (Roulston et al. 2003), the fight initiator, the number of interactions for the resident colony to kill the intruding worker, and the maximum number of workers cooperating to kill the intruder.

### Assay 4: resource competition in large arena

This assay was designed to predict the outcome of the behavioral interactions that might occur when *L. humile* and *T. sessile* compete at food sources under field conditions. For each species, colony fragments consisting of 500 workers, 10 queens, and *ca.* 50 brood were placed into plastic boxes ($19 \times 13 \times 9$ cm$^3$ high) and provided with a moist plaster nest (9 cm ∅). The ants were acclimated to their nests for 2 days without food. The boxes were connected to a central foraging arena ($70 \times 70 \times 5$ cm$^3$ high) by 10 ft of coiled plastic tubing (10 mm ∅). An empty nest (9 cm ∅) was placed in the center of the foraging arena. During the acclimation period the ants had access to the tubing, but not the foraging arena. On day 3, a 25 mL cup containing 20% sucrose solution was placed next to the nest in the foraging arena and the ants were given access to the foraging arena. We investigated competition for liquid carbohydrate food in two scenarios: (1) when both species were given access to the central arena at the same time, and (2) when *T. sessile* were allowed access to the central arena during the initial acclimation period, while *L. humile* was not. In the first scenario, both species are given an equal opportunity to explore the central arena and discover the food. In the second scenario, *T. sessile* had the advantage to explore the central arena, colonize the nest next to the food source, and possibly mark the arena with colony-specific chemicals to indicate home ranges and/or territory. Species-specific home range marking occurs in ants (Mayade et al. 1993; Jaffe et al. 1979) and the importance of a familiar territory for defense has been demonstrated in numerous studies (e.g., Hölldobler and Lumsden 1980; Gordon 1989). Each experiment was replicated four times. After the food was introduced, we monitored the colonies continuously to determine which species discovered the food first. Subsequently, we recorded daily the number of dead ants for each species and the species in possession of the sucrose solution. At the end of the test (day 6), we recorded the distribution of workers of both species, noting whether the ants nested in their own nest, the foraging arena nest, and/or the nest of the opposing species.

### Statistical analyses

All data analyses were performed using SAS 8.1 statistical software (SAS 2002). Differences in levels of aggression and mortality were compared using PROC TTEST. The PROC TTEST procedure examines the equality of variances and we report results of one of two types of *t*-tests, depending on the equality of variances. Results of a Student's *t*-test are reported when the variances were homogenous. In cases where the variances were unequal, we used the Welch *t*-test with a Satterthwaite correction (Zar 1999). Differences in the proportion of aggressive interactions initiated by the competing species were examined using Pearson's chi-square test.

## Results

### Assay 1: dyad interactions

One-on-one fights revealed high aggression between *T. sessile* and *L. humile* and the average maximum aggression score was 4.0 ± 0.0 SE. On average, the fights progressed through 13.2 ± 0.8 SE interactions before one of the ants was killed. Odorous house ants initiated 13/40 (33%) of fights and Argentine ants initiated 27/40 (66%) ($t = 5.17$, $df = 6$, and $P = 0.002$). Chi-square analysis of Table 1 revealed no association between the initiator of fights and the outcome of aggressive interactions (Pearson $\chi^2 = 2.57$, $df = 1$, and $P = 0.109$). Even though *T. sessile* initiated significantly fewer fights, they won 22/40 encounters (55%) and Argentine ants won 18/40 encounters (45%) ($t = -1.10$, $df = 6$, and $P = 0.315$). Both Argentine ants and odorous house ants used physical aggression and chemical defensive compounds against each other (Fig. 1A, B). Physical aggression was the primary defense mechanism for both species (Fig. 1A). Argentine ants used physical aggression in 34/40 (85%) interactions and odorous house ants used physical aggression in 36/40 (90%) interactions ($t = -1.00$, $df = 6$, and $P = 0.360$). Both species used physical aggression in 100% of encounters they won. Both species also used defensive chemicals (either alone or in conjuction with physical defenses), although less frequently. Argentine ants used chemical defenses in 24/40 (60%) interactions and odorous house ants used chemical defenses in 21/40 (53%) interactions ($t = 1.19$, $df = 6$, and $P = 0.279$). In the majority of one-on-one interactions, both species often used physical aggression and defensive chemicals simultaneously (Fig. 1B). Argentine ants used both defensive mechanisms in 23/40 (58%) interactions and odorous house ants used both defensive mechanisms in 21/40 (53%) interactions ($t = 0.74$, $df = 6$, and $P = 0.488$). Both species were also equally likely to use physical aggression alone or no aggression at all. Chemical defensive compounds were rarely used alone. The outcome of one-on-one interactions depended strongly on whether the ants used physical aggression and defensive chemicals simultaneously (Fig. 1C). Argentine ants used both defensive mechanisms in 16/18 (89%) interactions they won and odorous house ants used both defensive mechanisms in 18/22 (82%) interactions they won ($t = -1.00$, $df = 6$, and $P = 0.360$). In the majority of replicates, failure to use one of the defensive mechanisms by one of the species resulted in the other species winning the fight.

### Assay 2: symmetrical group interactions in a neutral arena

Symmetrical group interactions utilized an equal starting number of workers, 20 for each species. However, the fights quickly became asymmetrical as one species gained an advantage over the other. In contrast to one-on-one interactions, Argentine ants outcompeted odorous house ants in 20-on-20 interactions. At the end of the test (i.e., 8 h after mixing the two species) 94.6 ± 2.3% of odorous house ants and 68.3 ± 3.9% of Argentine ants were dead (Fig. 2; $t = -5.82$, $df = 18.1$, and $P < 0.0001$). The average

**Table 1** Contingency table indicating the outcome of aggressive interactions between *L. humile* and *T. sessile* in one-on-one interactions

| | | Fight initiator | | Total |
|---|---|---|---|---|
| | | *T. sessile* | *L. humile* | |
| Fight winner | *T. sessile* | 11/40 (27.5%) | 11/40 (27.5%) | 22/40 (55.0%) |
| | *L. humile* | 2/40 (5.0%) | 16/40 (40.0%) | 18/40 (45.0%) |
| Total | | 13/40 (32.5%) | 27/40 (67.5%) | 40/40 (100%) |

Numbers indicate the proportion of fights initiated by the intruding species ($n = 40$)

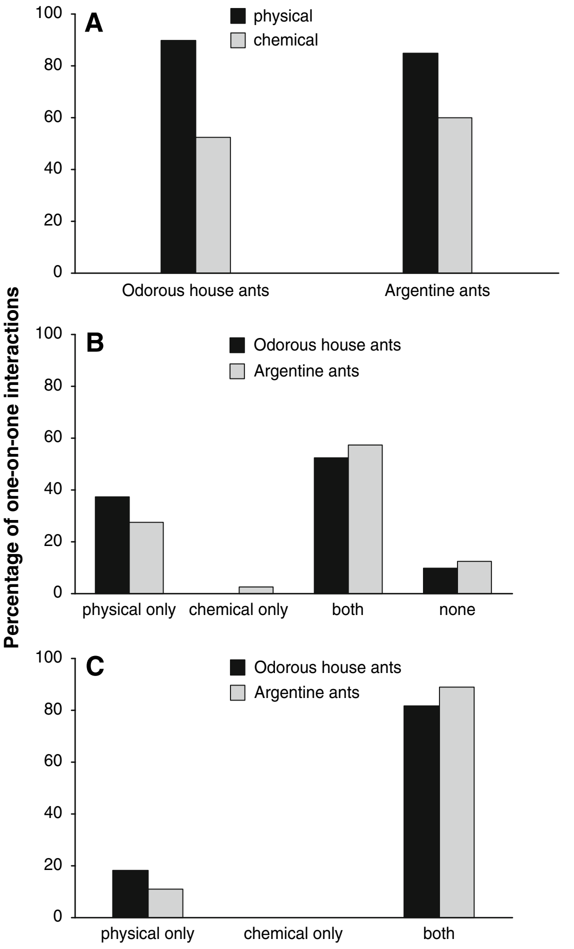

**Fig. 1** Results of the one-on-one interactions between Argentine ants and odorous house ants. (**A**) The frequency of use of physical defenses or chemical defenses, (**B**) the frequency of use of physical defenses alone, chemical defenses alone, a combination of both physical and chemical defenses, or lack of any defensive mechanism in all fights ($n = 40$), (**C**) the frequency of use of physical defenses alone, chemical defenses alone, a combination of physical and chemical defenses as a percentage of total fights won. No pairwise comparisons in Fig. 2A–C are significantly different ($\alpha = 0.05$)

ratio of odorous house ants to Argentine ants involved in fights was 0.6 ± 0.04% indicating that every odorous house ant worker involved in fights was attacked by ~1.7 Argentine ant workers.

## Assay 3: intruder introduction to resident territory

Intruder introduction assays demonstrated how colonies of *T. sessile* and *L. humile* react to heterospecific intrusions. Aggression toward intruders was always high, both when *L. humile* and *T. sessile* defended their territories. The average aggression score toward the intruders was 4.0 ± 0.0 SE, both when *T. sessile* were introduced into *L. humile* territory and vice versa. There were, however, pronounced differences in other aspects of the fights. It took odorous house ants significantly longer to kill intruding Argentine ants then it did Argentine ants to kill intruding odorous house ants. The average number of interactions to kill the intruder was 8.6 ± 0.8 SE when Argentine ants defended their territory. In contrast, it took odorous house ants 13.4 ± 0.4 SE interactions to kill intruding Argentine ants ($t = 4.06$, $df = 78$, and $P = 0.0001$). When *T. sessile* acted as intruders, they initiated 5/40 fights (12%). In contrast, when *L. humile* acted as intruders, they initiated 20/40 fights (50%) ($t = 4.39$, $df = 6$, and $P = 0.006$). This indicates that in comparison to odorous house ants, Argentine ants are likely to initiate fights even when they are in a foreign territory (Table 2).

We also determined that Argentine ant workers cooperate in fighting against odorous house ants, whereas odorous house ants usually fight alone and do not receive help from their nestmates. Argentine ants attacked odorous house ants in groups, whereby some workers pulled on the legs and/or the antennae of the intruders and other workers dismembered the intruder. Argentine ants fought in groups in 30/40 (75%) encounters, whereas odorous house ants rarely received help from their nestmates and fought collectively in only 6/40 (15%) interactions. On average, 3.2 ± 0.1 SE Argentine ant workers attacked an intruding odorous house ant worker (range 1–6 workers). In contrast, only 1.2 ± 0.1 SE odorous house ant workers attacked an intruding Argentine ant worker (range 1–2 workers) ($t = -7.41$, $df = 78$, and $P < 0.0001$).

## Assay 4: resource competition in large arena

Argentine ants clearly dominated all aspects of interactions with odorous house ants including access to the food and nests. Prior acclimation to the central arena did not give the odorous house ants any significant advantage over Argentine ants and we detected no difference in the average mortality of odorous house ants with (31.1 ± 5.5% SE) or without

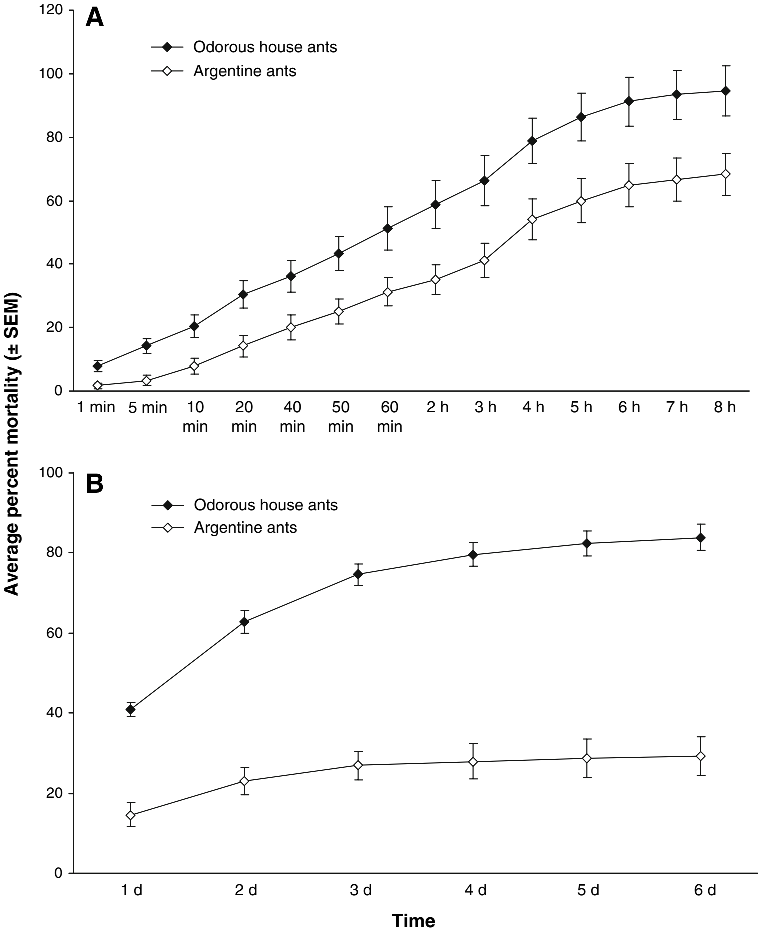

**Fig. 2** Mean percentage mortality (±SEM) in Argentine and odorous house ant workers in (**A**) symmetrical group interactions and (**B**) large arena assays

**Table 2** Contingency table indicating the outcome of aggressive interactions between *L. humile* and *T. sessile* in intruder introductions

| | | Fight initiator | | Total |
|---|---|---|---|---|
| | | *T. sessile* | *L. humile* | |
| Fight winner | *T. sessile* | 5/40 (12.5%) | 35/40 (87.5%) | 40/80 (50.0%) |
| | *L. humile* | 20/40 (50.0%) | 20/40 (50.0%) | 40/80 (50.0%) |
| Total | | 25/80 (31.5%) | 55/80 (68.5%) | 80/80 (100%) |

Numbers indicate the proportion of fights initiated by the intruding species ($n$ = 80)

(27.6 ± 3.9% SE) prior acclimation ($t$ = 0.57, $df$ = 6, and $P$ = 0.591). Therefore, for all subsequent analyses, we combined the data from the two sets of assays. On day 6, the average combined mortality was 83.9 ± 4.8% SE for *T. sessile* and 29.3 ± 3.2% SE for *L. humile* (Fig. 2; $t$ = –9.47, $df$ = 14, and $P < 0.0001$). *T. sessile* sustained the highest mortality during the first 2 days, when *L. humile* first explored the central arena and invaded *T. sessile* nesting space. The fighting subsided on day 3 and few ants fought by day 6. Odorous house ants suffered 100% mortality in 2/8 replicates. In the remaining six replicates, the two species partitioned the space, avoided interactions, and the mortality in *T. sessile* ranged from 57 to 88%.

When both species were given access to the central foraging arena simultaneously, Argentine ants always discovered the food first (4/4 replications) and dominated the resource for the duration of the test. When odorous house ants had the opportunity to acclimate to the foraging arena, they discovered the food first in 3/4 replicates, but lost possession within a day in all replicates. To determine which species controlled access to the food throughout the test we monitored the identity of ants (one, both, or no species feeding) daily. Argentine ants were present on the food in 32/48 (67%) of observations, odorous house ants in 3/48 (6%). The two species never occupied the feeding station at the same time and ants of either species were absent from the food in 13/48 (27%) of observations.

Argentine ants were also significantly more aggressive in colonizing empty nests and invading odorous house ant nests. In fact, *L. humile* invaded *T. sessile* nests in 8/8 replicates, whereas *T. sessile* never invaded *L. humile* nests. At the end of the test (day 6), Argentine ants nested in both the original nest and the center nest in 3/8 (38%) replicates, center nest only in 3/8 (38%) replicates, and in original nest, center nest, and *T. sessile* nest in 2/8 (25%) replicates. These results indicate that Argentine ants move nests closer to the food, while odorous house ants remain in their nests.

## Discussion

In fights involving groups of social animals, the winning group is likely to be numerically superior (Adams 1990; Traniello and Beshers 1991). Furthermore, according to Lanchester's laws of combat (Lanchster 1916), numerical strength must by supported by the individuals' willingness and ability to fight. In controlled arena assays, where the size of the opposing groups can be made equal, the importance of the individuals' willingness and ability to fight becomes especially critical. By increasing their willingness to fight as part of a group, individuals decrease the risk of injury or death as the likelihood of winning fights increases with increasing group size. Cooperative fighting by Argentine ants was evident in both 20-on-20 assays and intruder introductions. In 20-on-20 assays, an average of 1.7 Argentine ant workers attacked each odorous house ant worker. In intruder introduction assays, Argentine ants fought in groups in 30/40 (75%) encounters, whereas odorous house ants rarely received help from their nestmates and fought collectively in only 6/40 (15%) interactions. In the 20-on-20 assay *L. humile* lacked global numerical advantage, but won fights with *T. sessile* by creating local numerical advantage by attacking in groups. By fighting in groups and systematically killing off individual *T. sessile* workers, *L. humile* quickly created global numerical advantage and ultimately won battles with *T. sessile*. This suggests that the number of aggressive *L. humile* present (or functional group size), and not the absolute number, determines the fighting ability of *L. humile* colonies. Similar results were obtained by Tanner (2006) who conducted five-on-five aggression assays between *Formica xerophila* and *Formica integroides* and observed that a few *F. xerophila* would simultaneously engage a single *F. integroides*. Fighting in groups improved *F. xerophila's* competitive ability without increasing its risk of death. Furthermore, previous research demonstrates that fighting in groups allows Argentine ants to outcompete much larger and ecologically dominant ant species in areas where Argentine ants invade new areas (Human and Gordon 1996, 1999; Holway 1999).

Our results demonstrate a clear disparity between worker-level and colony-level fighting ability of Argentine ants and provide behavioral evidence to explain the superior interference ability of Argentine ants in group assays. Argentine ants experienced mixed success in fighting against odorous house ants in one-on-one interactions. This is consistent with the results by Holway (1999) who demonstrated that Argentine ants often lost one-on-one encounters with native ant species, including *T. sessile*. *L. humile* and *T. sessile* were equally likely to win one-on-one interactions, even though Argentine ants initiated 2/3 of all fights. This is not surprising, given that Argentine ants and odorous house ants are very similar in size and both species employ effective physical and chemical defenses. While body size is an important factor affecting the outcome of aggressive interactions among ants (Nowbahari et al. 1999), other factors, such as effective chemical defenses also play a role (Fellers 1987; Holway 1999). Indeed, previous studies demonstrate that Argentine ants often lose fights to much smaller ant species that possess highly repellent chemical defensive compounds (Holway 1999; Alder and Silverman 2005). Furthermore, our results demonstrate that using both physical and chemical defenses simultaneously is critical to winning fights by either species. Both species were equally likely to use both defensive mechanisms simultaneously and the failure to use one or both mechanisms usually allowed the opposing species to win.

Our observations indicate that two independent mechanisms facilitate wins by Argentine ants. First, Argentine ants cooperate in fighting against the opposing species, whereby some workers pull on the legs and/or the antennae of the opponents, while other workers dismember the opponent. In contrast, such behavior was rarely observed in odorous house ants, which almost always fought alone or avoided fights altogether. Second, Argentine ants seem to be immune to their own defensive chemicals, whereas odorous house ants appear to be highly repelled by each other's defensive chemicals, which may keep individual workers from teaming up against opponents. Whenever *T. sessile* encountered a fighting nestmate they became highly agitated, begun to run erratically, and away from the fighting pair. In *T. sessile*, the defensive chemicals also serve as the colony alarm pheromone, which may explain why *T. sessile* disperse upon encountering a fighting nestmate. This has profound implications in group fights, where several nestmates may be using defensive chemical simultaneously and in close proximity to each other. We observed that *L. humile* defensive chemicals are highly repellent to *T. sessile*; however, *L. humile* workers are not affected by each other's defensive chemicals. In fact, the release of defensive chemicals by a single worker may stimulate other workers to join the fights. In other eusocial insects (e.g., the honeybee), sting alarm pheromones release a defensive response from nearby workers (Gary 1975). Paradoxically though, when opposing Argentine ant colonies fight, they appear mutually affected by their defensive chemicals (Buczkowski and Silverman 2005).

When deciding whether or not to join a fight, a social individual faces a trade-off between the benefit of winning a fight and the cost of injury or death. According to the cost-minimizer hypothesis (Starks et al. 1998), nestmates are willing to share the cost of colony defense in groups, but not singly. As a result, an individual's willingness to enter a fight should increase as the size of the group increases. First, however, an individual must correctly assess group size and previous studies demonstrate that ants are able to assess group strength prior to encounters with a competitive species (Sakata and Katayama 2001; Tanner 2006). Ants that perceive themselves as part of a larger group act more aggressively toward a competitor than ants that perceive themselves as isolated individuals. To assess group size ants most likely rely on semiochemicals, specifically territory pheromones, which have been shown to control the aggressive behavior of ants (Mayade et al. 1993; Jaffe et al. 1979). Normally, higher concentrations of territory pheromones indicate that a larger number of nestmates is nearby and areas of high nestmate density usually indicate areas important for the colony, such as foraging trails and the nest. Previously, Buczkowski and Silverman (2005) showed that intraspecific aggression in the Argentine ant is context-dependent and occurs at higher rates when either nestmates or familiar territory indicate nest proximity. Context dependency of aggression 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. In interspecific dyadic interactions involving fights between *T. sessile* and *L. humile*, *L. humile* workers behave aggressively even in the absence of familiar territory and nestmates. This clearly indicates that Argentine ants perceive intraspecific competitors differently from interspecific competitors. In the 20-on-20 assay both species fought in a foreign territory, not previously marked with any species- or colony-specific cues. Lack of such cues would have prevented either species from making decisions about any numerical advantage as indicated by pheromone gradients, unless the ants use visual cues to assess group strength, which seems rather unlikely. Yet, *L. humile* fought in groups, while *T. sessile* rarely assisted nestmates being attacked by *L. humile* and usually fled upon detecting a fight in progress. The unexplored possibility is that in the absence of species-specific pheromones as indicators of group strength, defensive chemicals released by fighting ants may signal the presence of additional nestmates and stimulate other individuals to join the fights. Several studies have shown that in social organisms, an individual is more likely to be aggressive as the number of aggressive group members nearby increases (Wilson 1971; Sands 1982; Sakata and Katayama 2001), a process called social facilitation (Hölldobler and Wilson 1990). Our results indicate that *L. humile* may be subject to social facilitation, whereas *T. sessile* are not.

Given the similarities that *L. humile* and *T. sessile* share with respect to their foraging ecology, dietary preferences, diel feeding periodicity, and nesting preferences the two species may aggressively compete for food resources and nesting space where their geographic ranges overlap. Competition for food is an important factor shaping ant communities (Hölldobler and Wilson 1990) and there is considerable evidence that interspecific competition affects the distribution and abundance in ant communities, including those affected by Argentine ants (Human and Gordon 1996; Holway 1999). Our large arena assays demonstrate that *L. humile* clearly dominate all aspects of interactions with odorous house ants including speed of recruitment and control over food sources and nests. This is consistent with the results of previous field studies which indicate that *L. humile* depress the foraging success of native ants and ultimately lead to their displacement. Human and Gordon (1996, 1999) examined in field studies interference and exploitation competition by Argentine ants against native ant species and demonstrated that relative to Argentine ants, odorous house ants spent less time at baits, recruited fewer workers, gave up baits without fights, and were eventually displaced from 90% of all baits. Holway (1998) utilized pitfall traps to compare ant species diversity and abundance in areas with and without Argentine ants. Odorous house ants were one of the most dominant species in areas free of Argentine ants and were completely absent from areas invaded by Argentine ants. Competition for nests may also be a factor shaping interactions between *L. humile* and *T. sessile* given the similarity in their nesting preferences. In natural areas both species nest superficially in the soil and/or plant litter and seek human-made and protected harborages in urban areas. Given the propensity of both species to form large polygynous and polydomous supercolonies and engage in dispersed central-place foraging (Suarez et al. 1999; Holway and Case 2000; Buczkowski and Bennett 2006), the two species may actively compete for nests, especially where attractive nesting sites are in close proximity to food (e.g., at the base of trees housing Homoptera).

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