



# Effects of colony-level variation on competitive ability in the invasive Argentine ant

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Although colony size and nest number are believed to influence competitive ability in social insects, experimental studies testing this idea are rare. Here, we experimentally manipulated worker number and nest number in laboratory colonies of Argentine ants, *Linepithema humile*, to test how these attributes, working alone or in combination, affected different components of exploitative and interference ability. As expected, every measure of competitive performance tested increased with worker number. In contrast, the influence of nest number was more complex, with colony-level performance increasing, decreasing, or remaining constant depending on the type of competition-related test or colony-size category being considered. In the exploitation of randomly distributed food items, retrieval rates decreased with nest number for 50-worker colonies yet increased with nest number for 2500-worker colonies. In contrast, retrieval rates decreased with nest number across all colony sizes when resources were clumped. In two experiments on interference competition, only colonies with more than 1000 workers and that occupied single nests were able to maintain more than 10 workers, on average, at baits in the presence of a competitor, *Forelius mccooki*. Argentine ant workers initiated a majority of pairwise fights against *F. mccooki* workers but often lost (i.e. either retreated or were injured), in part because their pre-emptive attacks left them vulnerable to chemical defensive compounds used against them by *Forelius*. These findings promise to promote a better understanding of the causes of invasion success not only for the Argentine ant but for other invasive ants as well, given that most have colony structures resembling that of *L. humile*.

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Variation in body size has long been a prominent focus of ecology (Hutchinson & MacArthur 1959; MacArthur 1972; Peters 1983; Calder 1984; Brown 1995). Analyses of body size are particularly common in community ecology, in part because body size is easy to measure and is often correlated with characteristics important in determining ecological relationships (Brown 1995). For example, size is often a deciding factor in competitive interactions: large individuals typically dominate smaller ones (Stamps 1983; Gaudet & Keddy 1988). For modular, colonial and social organisms, however, competitive ability may correlate more with the size of the group than with the size of its individual components (Buss 1981). In this sense, attempts to understand the factors responsible for variation in group size are reminiscent of efforts to understand the factors responsible for variation in body size in unitary organisms.

In social insects, colony size is a key ecological variable (Michener 1964; Oster & Wilson 1978; Hölldobler &

Wilson 1990) that shows tremendous variation, both within (Tschinkel 1993) and among (Kaspari & Vargo 1995) species. Colony size influences both the foraging behaviour of individual workers (Gordon 1995; Herbers & Choiniere 1996) and the competitive prowess of entire colonies (Hölldobler 1981; Adams 1990). How colony size affects competitive ability, however, depends strongly on the spatial arrangement of workers in a colony. For example, many ant colonies (and those of some termites) maintain multiple nests, a condition known as polydomy, which may improve foraging efficiency (Hölldobler & Lumsden 1980; McIver 1991; Bourke & Franks 1995) and resource defence capabilities (Davidson 1997). Although recent theoretical work addresses the role of colony size in the functioning of social insect colonies (Pacala et al. 1996; Anderson & Ratneiks 1999), little is known about how colony size and nest number interact to influence different components of competitive performance.

Here we investigate how variation in these colony-level attributes influences competitive ability in the Argentine ant, *Linepithema humile*. This widespread invasive species

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displaces native ants throughout its introduced range (Newell & Barber 1913; Hölldobler & Wilson 1990; Majer 1994). In California, where this displacement is well documented, Argentine ants supplant most species of above-ground foraging ants (Erickson 1971; Tremper 1976; Ward 1987; Human & Gordon 1996, 1997; Holway 1998a, b; Suarez et al. 1998). Like other invasive ants (Hölldobler & Wilson 1977; Passera 1994), introduced populations of Argentine ants are unicolonial, forming expansive polydomous supercolonies that lack distinct behavioural boundaries (Newell & Barber 1913; Markin 1968, 1970; Suarez et al. 1999; Tsutsui et al. 2000). In part because introduced populations of Argentine ants typically do not defend territories against conspecifics, their colony sizes can greatly exceed those of native ants (Holway 1998a), helping to explain their ability to dominate native ants (Holway et al. 1998).

In this study, we experimentally manipulate worker number and nest number in laboratory colonies of Argentine ants to assess how each attribute affects different components of competitive performance. First, we focus on how these colony-level attributes influence the ability of colonies to exploit three classes of resources that differ in dispersion and composition. Second, we test how variation in worker number and nest number affect interference ability in the context of both resource defence and take-over against a native ant common in southern California, *Forelius mccooki*.

## METHODS

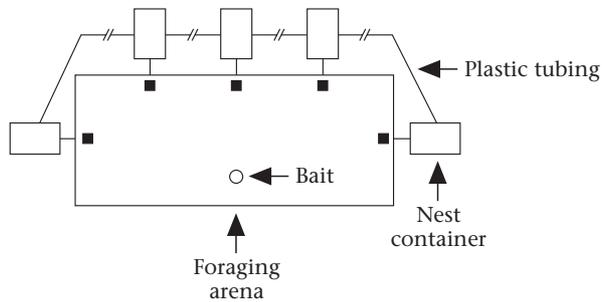
### Collection and Maintenance of Experimental Colonies

We collected source material for experimental colonies from a supercolony of *L. humile* located in La Jolla, San Diego Co., California, U.S.A. (32°50'N/117°12'W). After removal from the field, we divided supercolony fragments into 45 experimental colonies that differed both in worker number (50, 500 and 2500 workers) and nest number (one, three and five interconnected nest containers). We used a fully crossed, factorial design; each factorial combination contained five replicates. Nest containers were plastic boxes (30 × 14 × 8 cm) lined with fluon and tanglefoot to prevent ants from escaping. Each nest container had three nest chambers (glass test tubes: 16 × 150 mm) half full of water and stopped with cotton; we covered nest chambers with aluminium foil to keep them dark. For replicates with more than one nest container, we connected containers together using 3-m lengths of plastic tubing, which allowed workers in these replicates to move freely among nest containers. All replicates contained 10 queens. For replicates with three nest containers, we placed four queens in one of the containers and three queens in the other two. For replicates with five nest containers, we placed two queens in each container. In all polydomous replicates, we placed an aluminium foil barrier at the exit tube of each nest container to keep queens in their nest containers; each barrier had a small hole allowing the passage of workers (and workers carrying brood) but not the larger queens.

Approximately every other day, we fed experimental colonies scrambled eggs, *Drosophila*, crickets, vitamins and 25% sugar water. We reared colonies under standard laboratory conditions (12:12 h light:dark cycle at 24 °C). Before onset of experiments, we left experimental colonies in an unmanipulated state for 1 month.

The paucity of experimental studies examining how variation in colony-level attributes influences the competitive ability of social insects reflects the difficulty of conducting such experiments. In this study, we took advantage of the Argentine ant's unusually plastic colony structure and the ease with which this species cultures in the laboratory. Argentine ants thrive under laboratory conditions such as those described above and are commonly used in behavioural studies (Keller 1988; Deneubourg et al. 1990; Vargo & Passera 1991; Gordon 1995; Nonacs & Soriano 1998). Although our approach has obvious advantages, it is not without its limitations. The range of worker numbers and nest numbers employed here is a small and nonrandom subset of those found in nature. For example, the number of workers in individual nests from the field can greatly exceed the size of the experimental colonies used here and supercolonies as a whole may extend over many square kilometers (Newell & Barber 1913; Tsutsui et al. 2000). We intentionally chose to work with small colonies for reasons of feasibility and because we focused on small-scale, short-term aspects of exploitative and interference performance (see below), in which one to several nests would be involved in resource acquisition as opposed to entire supercolonies. Because the experimental colonies used here are biased towards the small end of colony-size distributions found in nature, the results of this study are particularly relevant to incipient infestations of Argentine ants and to colonies occupying abiotically marginal habitats (e.g. dry, exposed sites). In such cases, both worker number and nest number are likely to be smaller than for established colonies in favourable environments.

To test experimental colonies, we allowed them to forage in a rectangular foraging arena (Fig. 1). The arena had a smooth white floor and vertical stripping (4 cm high and lined with fluon) running along each edge to prevent ants from escaping. To provide experimental colonies with access to the arena, we placed bridges (bent 0.5 m lengths of wire fitted with clay feet) from each nest container to fixed access points in the foraging arena. The same access points were used for all experiments (Fig. 1): treatments with single nests were provided with one central access point; treatments with three nests were provided with three evenly spaced access points; and treatments with five nests were provided with five evenly spaced access points (Fig. 1). We conducted five separate experiments, each documenting a different aspect of exploitative or interference ability (see below). For each experiment, we tested experimental colonies in random order over a 2-week period. All experimental colonies were starved for approximately 48 h prior to testing. After each trial, we wiped the floor of the arena with ethanol to remove any residual recruitment pheromone and waited at least 30 min before starting the next trial.



**Figure 1.** Diagram of an experimental colony with five nest containers placed adjacent to the foraging arena (1.3×2.5 m). To provide experimental colonies access to the arena, we placed wire bridges connecting each nest container to its access point (filled squares) in the arena. Experimental colonies with a single nest container and those with three nest containers were arranged symmetrically with respect to the central access point shown in the figure. Access points remained fixed for all five experiments in this study. The location of the bait (either *Drosophila* or sugar water) remained fixed for experiments 2–5. Plastic tubing (3 m lengths) connected nest containers in polydomous replicates. Figure not drawn to scale. See Methods for additional details.

The five experiments in this study were conducted over a 7-month period. During this time, colony sizes did not remain constant. Experimental colonies with 50 workers at the start of the experiment grew in size to an average of 204 workers by the end of the experiment. The productivity of these small colonies agrees with the results reported by Hee et al. (2000). In that study, per capita growth rates were highest in colonies with the fewest workers. Experimental colonies that started with 500 workers remained around the same size, on average, with 543 workers by the end of the experiment, but 2500-worker colonies shrank in size to an average of 1532 workers at the end of the experiment. Because the size of all experimental colonies was known at the beginning and at the end of the study, we estimated colony sizes through linear interpolation for the time periods during which each of the five experiments was conducted. For experiments 1–3, which were conducted within 2 months of the establishment of experimental colonies, estimated colony sizes within each size category did not overlap with adjacent size categories. A 3-month period separated the timing of experiments 1–3 and experiments 4–5; during this time some of the colonies grew or shrank in size to the point that they overlapped in size with adjacent size categories. For experiments 4–5, we adjusted size categories to reflect differential colony growth and reclassified colonies to different size categories as needed. The revised size categories were as follows: 50–200 workers, 250–900 workers and more than 1000 workers. These categories corresponded with natural breaks in the distribution of estimated colony sizes. Using these new size categories, it was necessary to reclassify 9% of colonies in experiment 4 and 24% of colonies in experiment 5. (Sample sizes based on these adjustments are listed in the legend to Fig. 4.) Although colonies changed in size through the course of this study, replicates with more than one nest container remained functionally polydomous throughout: workers and queens were distributed

among nest containers and workers commonly moved among nests.

## Experiments on Exploitative Ability

### *Experiment 1: exploitation of dispersed, solid food*

In this experiment, we measured the ability of each experimental colony to harvest dead adult *Drosophila* scattered at random throughout the foraging arena. This experiment models exploitation of a dispersed resource harvested by solitary foragers because individual *Drosophila* are small enough for single Argentine ant workers to carry and the retrieval of scattered flies does not involve recruitment of nestmates to a specific location. We gridded the foraging arena into 100 squares (each square 15 × 11.5 cm); a buffer of 5–10 cm separated the grid from the edge of the arena. For each run of this experiment, we randomly selected 25 squares and placed a single *Drosophila* in the centre of each square. We then provided an experimental colony with access to the arena and counted the number of flies removed within 30 min (the dependent variable used in the analysis).

### *Experiment 2: exploitation of clumped, solid food*

This experiment was similar to experiment 1, except that *Drosophila* were clumped rather than dispersed. This experiment emulates exploitation of a resource too large for one worker to carry (e.g. a carcass of a large arthropod or small vertebrate) and for which recruitment of nestmates is necessary for efficient exploitation. For each run of this experiment, we placed 25 *Drosophila* together in a small pile at a fixed location opposite the central access point for all experimental colonies (Fig. 1). We then provided an experimental colony with access to the arena, measured the time required for it to locate the pile of flies, and counted the number removed within 15 min after discovery (the dependent variable used in the analysis).

### *Experiment 3: exploitation of clumped, liquid food*

This experiment was similar to experiment 2, except that we used a point source of sugar water. This experiment models the retrieval of an immobile resource, such as extrafloral nectaries or an aggregation of honeydew-excreting Homoptera. For each run of this experiment, we provided 0.4 g of a 25% solution of sucrose in water (sugar water) at a fixed location opposite the central access point for all experimental colonies (Fig. 1). Sugar water was presented in a single, large circular drop placed in a weigh boat (4 × 4 cm). We then provided an experimental colony with access to the arena, determined the time required for it to locate the sugar water, and measured the amount removed (corrected for evaporation) within 30 min after discovery (the dependent variable used in the analysis).

## Experiments on Interference Ability

### *Experiment 4: resource defence*

This experiment was similar to experiment 3, except that *Forelius mccoeki* workers were allowed to forage in the

vicinity of the sugar water after Argentine ants had discovered the bait. This experiment emulates a case of resource defence, where the first colony to discover a resource must recruit nestmates to it and defend it from other ants in order to retain possession. For each run of this experiment, we placed 0.4 g of 25% sugar water at a fixed location opposite the central access point for all experimental colonies (Fig. 1). In addition, we placed 50 workers of *F. mccooki* in the grid square adjacent to the sugar water; these workers were contained inside an inverted, circular (10 cm diameter) plastic container, both sides of which were lined with fluon to prevent ants from climbing on the container either from the inside or the outside. The lip of the container (adjacent to the floor of the arena) had a small notch cut into it allowing *Forelius* workers to exit; this notch remained blocked with a small piece of clay until the start of the experiment. After placement of sugar water, we gave an experimental colony of Argentine ants access to the foraging arena. Ten minutes after workers from the Argentine ant colony discovered the sugar water, we unplugged the opening in the container holding *Forelius* workers allowing them to exit, which they did at a rate of one to five per min. For the next 30 min, we counted the number of Argentine ant workers at the sugar water every 5 min. For analysis, the dependent variable was the number of Argentine ant workers present at the sugar water averaged across six 5-min counts starting 5 min after *Forelius* workers were first allowed out of their container. We also quantified the outcome of interactions between individual Argentine ant workers and individual *Forelius* workers. For each run of this experiment, we recorded up to five one-on-one behavioural interactions. For each interaction, we recorded the initiator, the apparent mechanism employed by both species (e.g. physical aggression or chemical defensive compounds), and the victor. Workers were considered victors if they caused their adversary to retreat greater than 5 cm from the location of the fight or if they unilaterally injured their opponent. The use of chemical defensive compounds is known for both Argentine ants (Lieberburg et al. 1975; Holway 1999) and *Forelius* (formerly *Iridomyrmex*) (Hölldobler 1982) and involves a stereotyped set of behaviours shared in common by both groups. For data analysis, each one-on-one interaction was considered a datum and observations for each trial were pooled across replicates within each experimental category.

#### Experiment 5: resource take-over

In this experiment, we reversed the dynamics of the interaction described in the above experiment by providing whole colonies of *F. mccooki* with access to a point source of sugar water before Argentine ants entered the foraging arena. This experiment models a case of resource take-over in which a colony must drive off workers from another colony in order to control possession of a resource. For each run of this experiment, we placed 0.4 g of 25% sugar water at a fixed location opposite the central access point for all experimental colonies (Fig. 1). We placed a *F. mccooki* colony (ca. 500 workers and 10 queens) in the grid square adjacent to the sugar water;

this colony was contained inside an inverted, rectangular (12 × 20 cm) plastic container, both sides of which were lined with fluon to prevent ants from climbing on the container either from the inside or the outside. The lip of the inverted container had a small notch cut into it allowing *Forelius* workers to exit. At the start of the experiment, we let *Forelius* forage outside of their container. Twenty minutes after *Forelius* first discovered the sugar water, we gave an Argentine ant colony access to the arena. We waited until a worker from the Argentine ant colony had discovered the sugar water, allowed this colony 10 min to recruit, and then counted the number of Argentine ant workers at the sugar water every 5 min for a 30-min period. For analysis, the dependent variable was the number of Argentine ant workers present at the sugar water averaged across six 5-min counts starting from 15 min after discovery. We also recorded data on one-on-one behavioural interactions, as in experiment 4. We collected the 10 laboratory colonies of *F. mccooki* used in this experiment at the University of California Elliot Chaparral Reserve (San Diego). Given that there were 45 experimental colonies of Argentine ants, we used each *Forelius* colony in four to five trials. Although each *Forelius* colony, on average ( $\pm$  SE), lost  $44 \pm 11$  workers during the course of this experiment, repeated use did not appear to affect the behaviour or activity of these colonies. We always waited at least 48 h before reusing a *Forelius* colony.

#### Statistical Analysis

For each of the five experiments, we compared measures of competitive ability across experimental groups using two-factor analysis of variance (ANOVA). For all ANOVAs, we treated both worker number and nest number as fixed factors because the levels used were set by us at the start of the experiment and were a nonrandom subset of those found in nature (see above). All dependent variables were  $\ln(x+1)$  transformed prior to analysis to homogenize variances. Within each ANOVA, variances were not different from one another after data were transformed (Cochran's test: NS). Following each ANOVA, we used Scheffe's tests to compare means across experimental categories. Although Scheffe's tests control the familywise error rate (Day & Quinn 1989), they do not control the experimentwise error rate. To maintain an experimentwise error rate of  $\alpha=0.05$ , *P* values for the Scheffe's tests were Bonferroni adjusted depending on the number of separate tests conducted within each ANOVA as suggested by Underwood (1997). In experiments 4–5, we also collected data on behavioural interactions between individual Argentine ant workers and individual *Forelius* workers. For each of the four kinds of data collected, that is, initiator, victor, frequency of physical aggression (i.e. lunging) and the frequency with which chemical defensive compounds were used, we used heterogeneity chi-square analysis to determine if pooling data across experimental groups was justified. For each class of data in each of the two experiments, chi-squares were homogeneous. Therefore, we pooled data across

**Table 1.** Analysis of variance tables for the five experiments conducted in this study

	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i> value	<i>P</i> value
<b>Exploitative ability</b>					
Experiment 1: Retrieval of dispersed, solid food					
Workers	2	29.847	14.924	94.509	<0.001
Nests	2	0.930	0.465	2.946	0.065
Interaction	4	3.815	0.954	6.040	0.008
Residual	36	5.685	0.158		
Experiment 2: Retrieval of clumped, solid food					
Workers	2	29.027	14.514	27.711	<0.001
Nests	2	6.695	3.347	6.391	0.004
Interaction	4	3.548	0.887	1.693	0.173
Residual	36	18.855	0.524		
Experiment 3: Retrieval of clumped, liquid food					
All colonies					
Workers	2	83.318	41.659	36.520	<0.001
Nests	2	2.466	1.233	1.081	0.350
Interaction	4	1.582	0.396	0.347	0.845
Residual	36	41.067	1.141		
Small colonies excluded					
Workers	1	7.196	7.196	22.436	<0.001
Nests	2	3.699	1.849	5.766	0.009
Interaction	2	0.349	0.175	0.544	0.587
Residual	24	7.698	0.321		
<b>Interference ability</b>					
Experiment 4: Resource defence					
Workers	2	6.891	3.446	56.920	<0.001
Nests	2	0.596	0.298	4.922	0.013
Interaction	4	0.171	0.043	0.706	0.593
Residual	36	2.179	0.061		
Small colonies excluded					
Workers	1	2.859	2.859	37.697	<0.001
Nests	2	0.414	0.207	2.729	0.085
Interaction	2	0.124	0.062	0.814	0.454
Residual	25	1.896	0.076		
Experiment 5: Resource take-over					
Workers	2	3.110	1.555	45.565	<0.001
Nests	2	0.590	0.295	8.644	0.001
Interaction	4	0.333	0.083	2.438	0.065
Residual	36	1.229	0.034		
Small colonies excluded					
Workers	1	1.576	1.576	39.174	<0.001
Nests	2	0.804	0.402	9.993	<0.001
Interaction	2	0.052	0.026	0.649	0.531
Residual	25	1.006	0.040		

experimental groups for each experiment. For each chi-square, the null hypothesis was that observed frequencies of a specific outcome or behaviour for the two species did not differ from equality.

## RESULTS

### Experiments on Exploitative Ability

Experiment 1 tested the ability of colonies to retrieve randomly scattered *Drosophila*. Because the interaction between worker number and nest number was significant (Table 1; ANOVA:  $F_{4,36}=6.04$ ,  $P<0.01$ ), we analysed colony-level performance for each level of each factor separately. For each nest-number category, retrieval rates increased with worker number, although 500-worker and

2500-worker colonies did not differ statistically from one another (Table 2). Results for each colony-size category revealed a more complex pattern. For 50-worker colonies, retrieval rates decreased with nest number: colonies with a single nest retrieved more flies during the experiment than did colonies with three nests (Fig. 2a; but note that the performance of colonies with five nests was intermediate between the performance of colonies with one nest and that of colonies with three nests). In contrast, retrieval rates for 2500-worker colonies increased with nest number: colonies occupying single nests retrieved fewer flies during the experiment than did colonies occupying five nests (Fig. 2a). For 500-worker colonies, nest number did not influence retrieval rate (Fig. 2a).

Experiments 2–3 tested the ability of colonies to retrieve two different resources, *Drosophila* and sugar

**Table 2.** Treatment means for retrieval rates (number of *Drosophila* retrieved within 30 min) and multiple comparisons (Scheffe's tests) for results of two-factor ANOVAs for experiment 1†

		Colony size		
		50 workers	500 workers	2500 workers
One nest				
Mean		1.823	2.438	3.090
Difference	50–500	–0.615	500–2500	–0.652
	50–2500	–1.267*		
Three nests				
Mean		0.497	2.728	3.132
Difference	50–500	–2.231*	500–2500	–0.404
	50–2500	–2.635*		
Five nests				
Mean		1.318	2.622	3.226
Differences	50–500	–1.304*	500–2500	–0.604
	50–2500	–1.908*		

\* $P < 0.05$ .†Due to the significant interaction in this ANOVA (Table 1), multiple comparisons were performed on each level of each factor separately. See also Fig. 2a. Data  $\ln(x+1)$  transformed.

water, that were clumped in space rather than dispersed. For both resources, retrieval rates subsequent to discovery increased with worker number but decreased with nest number (Table 1, Fig. 2b, c). Fifty-worker colonies were excluded from the analysis of the results of experiment 3 because they did not recruit to sugar water. When discovery time was incorporated into these analyses, the results were qualitatively similar. In experiments 2–3, the finding that retrieval rates decreased with nest number (Fig. 2b) contrasts to the one obtained in experiment 1 where, at least for 2500-worker colonies, retrieval rates increased with nest number (Fig. 2a). In experiment 3, the amount of sugar water removed increased linearly with the maximum number of workers present at the bait during the experiment (Fig. 3; linear regression:  $F_{1,43} = 249.17$ ,  $P < 0.0001$ ).

### Experiments on Interference Ability

Experiments 4–5 tested the ability of colonies to engage in two different aspects of interference competition: resource defence and resource take-over. As in the experiments on exploitative ability, colony-level performance increased with worker number (Table 1, Fig. 4a, b). Numerical advantages were key to the Argentine ant's ability to persist at baits in the presence of *Forelius*. This was especially evident in experiment 5 where 500-worker colonies of *Forelius* were given access to the sugar water before Argentine ants. Only large (>1000 workers) colonies of Argentine ants were able to maintain more than five workers, on average, at the bait (Fig. 4b). As in experiment 3, colonies in the smallest worker-number category were excluded from these analyses because they did not recruit to sugar water. Nest number also influenced colony-level performance. In both experiments, colonies with more than 1000 workers and that occupied single nests maintained the highest number of workers, on average, at the sugar water (Fig. 4a, b). The effect of

nest number was significant in experiment 5 but only marginally so ( $P = 0.085$ ) in experiment 4 (Table 1). Given the relationship between rate of resource retrieval and the number of workers present at the sugar water (Fig. 3), the results of these experiments show how both colony size and nest number affect resource retrieval in the face of interference competition from another species.

In both experiments 4 and 5, Argentine ant workers and *Forelius* workers fought aggressively with one another. Nearly all of these interactions took place between pairs of workers. Figure 5 summarizes the frequencies with which the two species initiated and won pairwise fights as well as the prevailing mechanisms used in these encounters. The results for experiment 4 (Fig. 5a) were similar to those for experiment 5 (Fig. 5b). Compared to *Forelius*, Argentine ants initiated but lost a majority of fights. The most common sequence of behaviours involved an Argentine ant worker lunging at a *Forelius* worker and attempting to grab a leg or antenna. Such attacks left Argentine ant workers vulnerable to the common countermeasure of *Forelius* workers, which involved rapidly turning in place so that chemical defensive compounds stored in the gaster could be employed. If an Argentine ant made contact with these compounds, it immediately retreated backwards and tried to clean its antennae and mouthparts. When *Forelius* lunged first, this behavioural sequence was often reversed. Although Argentine ants lost a majority of one-on-one interactions, the mortality inflicted on both sides during the course of each trial was more or less equal (Fig. 6).

### DISCUSSION

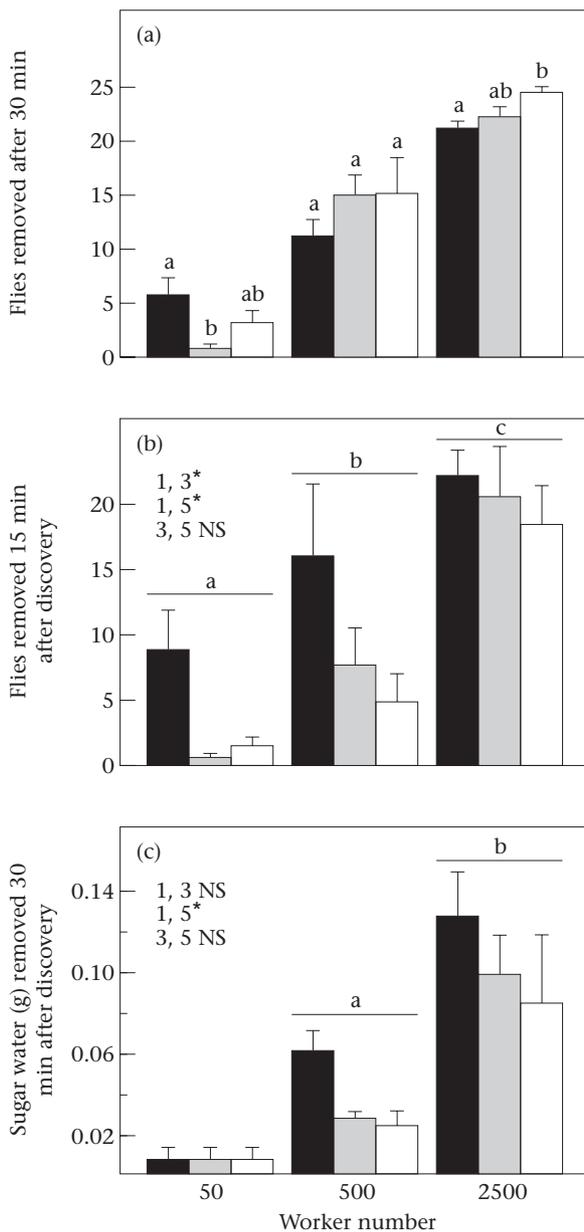
Although colony size (Hölldobler 1981; Hölldobler & Lumsden 1980; Adams 1990; Franks & Partridge 1993) and, to a lesser extent, colony spatial structure (Hölldobler & Lumsden 1980; McIver 1991; Traniello & Levings 1986; Davidson 1997) are believed to determine

competitive ability in many species of ants, few experimental studies have tested how these factors influence different aspects of competition-related performance. Our results demonstrate how variation in worker number and nest number affects both exploitative and interference ability. Not surprisingly, every measure of competitive ability examined increased with worker number. In contrast, the influence of nest number was more complex with colony-level performance increasing, decreasing, or remaining constant depending on the type of competition-related test or, in one case, colony-size category being considered. These dynamics are perhaps best illustrated with respect to resource exploitation. In the retrieval of randomly scattered *Drosophila*, colony-level performance decreased with nest number for small colonies but increased with nest number for large colonies. In the exploitation of scattered food items, polydomy

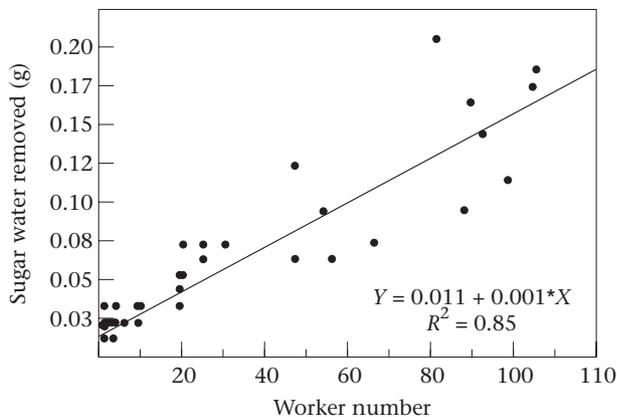
may be an asset for large colonies because workers are stationed in nests dispersed widely in space, effectively blanketing a colony's foraging area (Hölldobler & Lumsden 1980; Traniello & Levings 1986; Pfeiffer & Linsenmair 1998). For small colonies, however, polydomy may hinder foraging success if not enough workers are available to allow individual nests to function efficiently. In contrast to the exploitation of scattered resources, retrieval rates for clumped resources (*Drosophila* and sugar water) decreased with nest number. For a given colony size, colonies with single nests excelled in the exploitation of clumped resources apparently because of their superior ability to recruit workers to a single location. This capability probably stems from a variety of causes including a greater availability of recruits inside the nest (Johnson et al. 1987) and improved recruitment efficiency (Anderson & Ratneiks 1999).

In experiment 3 (see also experiments 4–5), 50-worker colonies failed to recruit workers to sugar water. There are three likely explanations. First, at least compared with large colonies, small colonies exploited food much less quickly. Second, because small colonies have few workers but high rates of per capita brood production (Hee et al. 2000), their nutritional demands for protein (required for brood production) probably exceed their demands for carbohydrates. Lastly, these colonies may have been too small to function normally. This seems unlikely given that 80% of the colonies in the smallest size class more than doubled in size during the course of the experiment. Furthermore, colonies of this size did retrieve food in experiments 1–2 in this study and have been used in other studies of the foraging behaviour of this species (Nonacs & Soriano 1998).

The advantages of exceptional recruitment abilities not only applied to resource exploitation but to interference competition as well. For both resource defence and takeover, colonies with more than 1000 workers and that



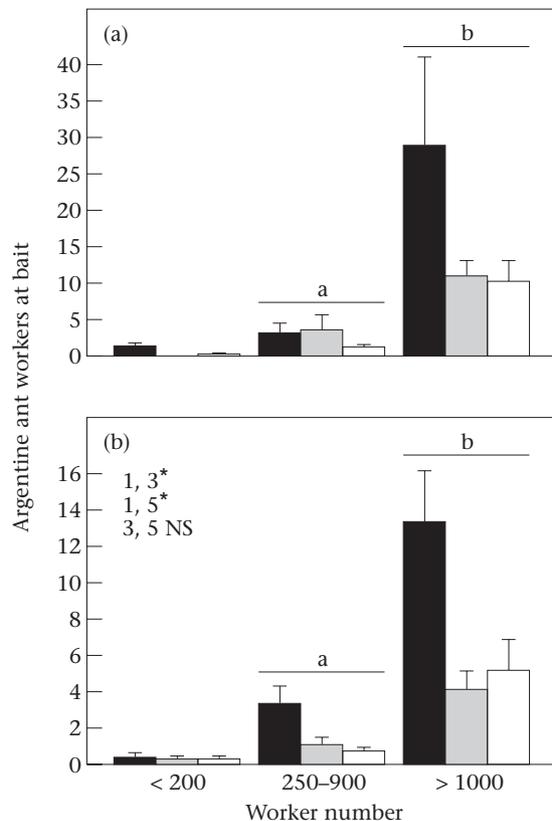
**Figure 2.** Colony-level performance in three measures of resource exploitation for colonies differing in worker number and nest number (■: 1 nest; □: 3 nests; □: 5 nests). For ANOVA tables, see Table 1. (a) Mean+1SE number of randomly dispersed *Drosophila* removed from the foraging arena within 30 min. Due to a significant interaction between worker number and nest number, multiple comparisons were made for each level of each factor separately (only those for the three levels of worker number are shown here; also see Table 2). For each worker-number category, bars with different letters were significantly different ( $P < 0.05$ ) from one another. Because of the interaction, the notation for (a) differs from that for (b) and (c), in which no interaction was present. (b) Mean+1SE number of *Drosophila* removed from a point source in the foraging arena 15 min after discovery. Trios of bars with different letters were significantly different ( $P < 0.05$ ) from one another. The inset shows patterns of significance ( $P < 0.05$ ) in resource retrieval rates as a function of nest number. (c) Mean+1SE amount (g) of sugar water removed from a point source in the foraging arena 30 min after discovery. Fifty-worker colonies were excluded from this analysis because they did not recruit to the sugar water. Trios of bars with different letters were significantly different ( $P < 0.05$ ) from one another. The inset shows patterns of significance ( $P < 0.05$ ) in resource retrieval rates as a function of nest number.



**Figure 3.** Regression showing the relationship between the amount of sugar water removed by Argentine ant workers 30 min after discovery and the maximum number of workers present at the bait during this interval.

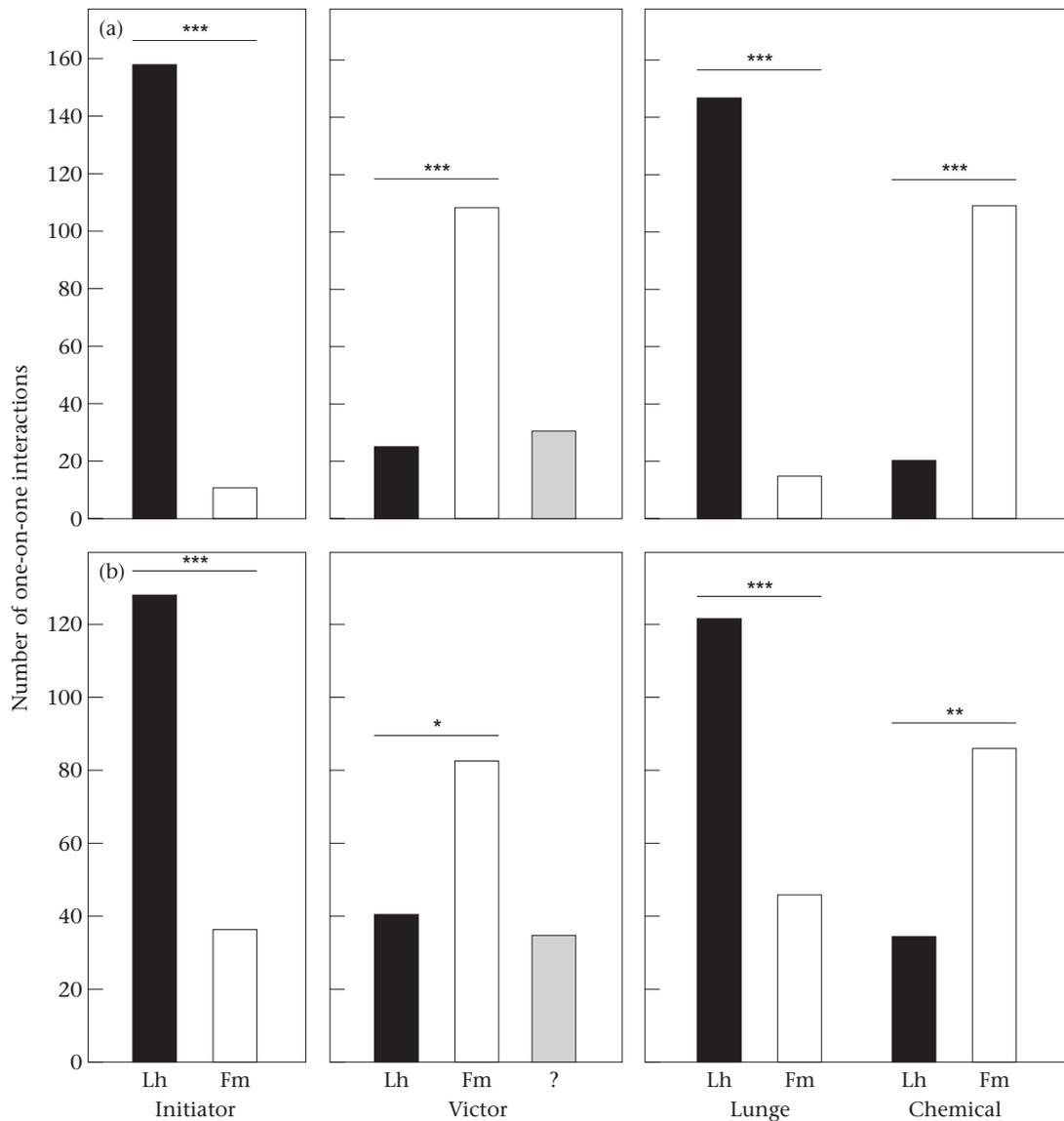
occupied single nests maintained the highest numbers of workers at the sugar water. As in the above experiments, colony size is important because it governs the size of a colony's recruitment response, which often decides the outcome of competitive interactions between opposing colonies (Lynch et al. 1980; Fellers 1987; Adams 1990). The significance of nest number in experiment 5, as in experiments 2–3, again apparently reflects the importance of superior recruitment. These experiments also illustrate that the Argentine ant's proficiency at interference competition against *Forelius* hinges on numerical advantages. For example, in experiment 5 only colonies with more than 1000 workers were able to maintain more than five workers, on average, at baits in the presence of *Forelius* colonies containing 500 workers. Although the effect of colony size on interference ability could depend on factors other than recruitment per se, Argentine ants from large colonies were no more likely to initiate or to win pairwise fights against *Forelius* than were workers from small colonies. Additional support for the importance of numerical superiority came from observations of one-on-one interactions and measures of direct mortality. Argentine ants initiated a majority of pairwise fights (see also Human & Gordon 1999) but typically lost, in large part because their pre-emptive lunges left them vulnerable to chemical defensive compounds used against them by *Forelius* (see also Hölldobler 1982). Despite that Argentine ants usually lost one-on-one fights, mortality incurred was split more or less evenly between the two species, indicating that *Forelius* and Argentine ants were approximately evenly matched, at least with respect to the number of workers dying during an experimental run. Taken together, these results show that numerical asymmetries are required for Argentine ants to gain the upper hand in contests against *Forelius*.

The findings of this study offer insight into the underlying causes of the Argentine ant's widespread success as an invader. In part because introduced populations of Argentine ants typically lack intraspecific aggression and, as a consequence, do not defend territories, their colony sizes can increase to levels exceeding those of native ants



**Figure 4.** Colony-level performance in two measures of interference competition for colonies differing in worker number and nest number (■: 1 nest; ▒: 3 nests; □: 5 nests). For ANOVA tables, see Table 1. For both (a) and (b), colonies with fewer than 200 workers were excluded from analyses because they did not recruit to the sugar water. (a) Mean+1SE number of Argentine ant workers present at a sugar water bait in which 50 workers of *Forelius mccooki* were also given access to the bait but 10 min after discovery by Argentine ants. This scenario emulates resource defence on the part of Argentine ants; see Methods for details. Trios of bars with different letters were significantly different ( $P < 0.05$ ) from one another. Sample sizes for this analysis were as follows: one nest, 250–900 workers ( $N=6$ ); one nest, more than 1000 workers ( $N=3$ ); three nests, 250–900 workers ( $N=4$ ); three nests, more than 1000 workers ( $N=5$ ); five nests, 250–900 workers ( $N=8$ ); five nests, more than 1000 workers ( $N=3$ ). (b) Mean+1SE number of Argentine ant workers present at a sugar water bait in which a 500-worker colony of *F. mccooki* was given access to the bait before Argentine ants. This scenario emulates resource take-over by Argentine ants; see Methods for details. Trios of bars with different letters were significantly different ( $P < 0.05$ ) from one another. The inset shows patterns of significance ( $P < 0.05$ ) in resource retrieval rate as a function of nest number. Sample sizes for this analysis are as follows: one nest, 250–900 workers ( $N=4$ ); one nest, more than 1000 workers ( $N=5$ ); three nests, 250–900 workers ( $N=4$ ); three nests, more than 1000 workers ( $N=6$ ); five nests, 250–900 workers ( $N=7$ ); five nests, more than 1000 workers ( $N=5$ ).

(Holway 1998a; Holway et al. 1998). These numerical advantages are believed to be key to explaining the competitive strength of the Argentine ant, given (1) that individual workers are small (2–3 mm in length) and often lose one-on-one confrontations against other

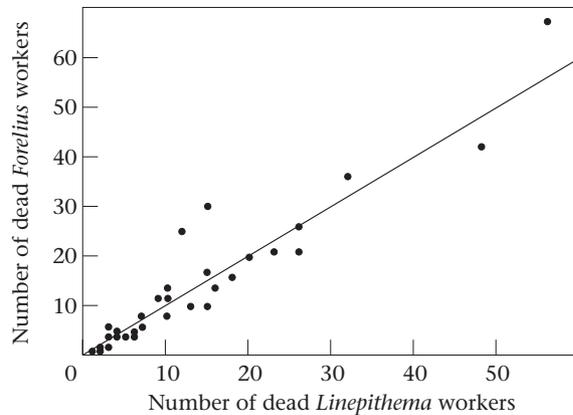


**Figure 5.** The frequency of behaviours observed in pairwise interactions between individual Argentine ant workers (Lh) and individual *Forelius* workers (Fm) for (a) experiment 4 and (b) experiment 5. Workers were considered victors if they caused their adversary to retreat more than 5 cm from the location of the fight or if they unilaterally injured their opponent. For some fights, it was not apparent which ant won; such interactions are placed in the '?' category. Both lunging and use of chemical defensive compounds involve a stereotyped set of behaviours shared in common by both species. For each of the four classes of data, we used chi-square tests to compare frequencies between the two species. Categories of significance for these analyses are as follows: \* $P < 0.01$ ; \*\* $P < 0.001$ ; \*\*\* $P < 0.0001$ .

species of ants (Tremper 1976; Holway et al. 1998; Holway 1999), and (2) that numerical superiority often decides the outcome of interference contests between colonies of ants generally (Hölldobler & Lumsden 1980; Adams 1990; Hölldobler & Wilson 1990). Here, we provide the first direct experimental evidence that the Argentine ant's ability to engage in interference competition depends on numerical advantages. It seems likely that the advantages of large colony size extend to exploitative ability as well. In this study, all aspects of exploitative performance increased with colony size. In addition to retrieving food quickly, large colonies also should enjoy advantages in terms of resource discovery, relative to small colonies, if they have

more workers in the field looking for food (Johnson et al. 1987).

Polydomy is an additional consequence of unicoloniality that may promote competitive strength. The maintenance of multiple nests may facilitate the exchange of resources, workers, brood and queens among nests, giving supercolonies tremendous flexibility to respond to spatio-temporal heterogeneity in resource availability (Holway & Case 2000). In contrast to the results on colony size, however, the results of this study provide only weak evidence that polydomy enhances the Argentine ant's competitive ability. Polydomy appeared advantageous only for large colonies retrieving dispersed resources; for all other tests, the degree of polydomy actually hindered



**Figure 6.** Scatterplot showing mortality from the experiment on resource take-over involving whole colonies of both Argentine ants and *Forelius mccooki*. The line is unity.

competitive performance. These results may not extend readily to field situations, given that the relative advantages of polydomy clearly depend on the size and spatial arrangement of nests. In this study, the placement of nests was fixed, nests remained close together, and a relatively small foraging arena was used (Fig. 1). None the less, the putative advantages of polydomy in terms of rate of retrieval of scattered versus clumped resources as reported here are consistent with findings reported in field studies (Hölldobler & Lumsden 1980; Traniello & Levings 1986; Pfeiffer & Linsenmair 1998). At least in theory, Argentine ant supercolonies could adjust the distribution of workers among nests to optimize colony-level efficiency with respect to both the distribution of resources and competitors. Although such flexibility probably contributes to the Argentine ant's competitive strength, it may also come at a cost. Argentine ants invest little in their ephemeral nests, which are typically in shallow soil, and may be less buffered from unsuitable abiotic conditions (e.g. aridity, cold) than the colonies of species that nest deeper in the soil. In Mediterranean California, appropriate physical conditions appear to govern both the distribution (Tremper 1976; Ward 1987) and spread (Holway 1995; Holway 1998b; Suarez et al. 1998) of the Argentine ant.

The findings of this study also may help explain the success of other invasive social insects. For example, many highly invasive ants display unicoloniality to varying degrees, occupying polydomous supercolonies with populous worker forces (Hölldobler & Wilson 1977; Hölldobler & Wilson 1990; Passera 1994). Examples include the following: *Pheidole megacephala* (Hoffmann et al. 1999), *Wasmannia auropunctata* (Clark et al. 1982) and the polygyne form of *Solenopsis invicta* (Porter et al. 1988; Porter & Savignano 1990). It will be of great interest to determine whether these species achieve competitive dominance in ways similar to those described for Argentine ants (Holway et al. 1998; Holway 1999; Holway & Case 2000). Experimental tests, such as the one described here (also see Morrison 1999, 2000), promise to clarify the causes of the strong competitive asymmetries between invasive ants and the native species they displace.

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