

Research article

Evidence that queens do not influence nestmate recognition in Argentine ants

E. J. Caldera¹ and D. A. Holway^{2,*}

¹ Department of Integrative Biology, University of Texas, Austin, TX, USA, e-mail: erock@mail.utexas.edu

² Section of Ecology, Behavior, and Evolution, Division of Biological Sciences, University of California at San Diego, La Jolla, CA 92093-0116, USA, e-mail: dholway@ucsd.edu

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Summary. Introduced populations of many invasive ants exhibit low levels of intraspecific aggression. Argentine ants (*Linepithema humile*), for example, maintain expansive supercolonies in many parts of their introduced range. Recent studies demonstrate that variation in nestmate recognition in *L. humile* can derive from both environmental and genetic sources. In some ants, pheromones emitted by queens also influence nestmate-recognition behavior. To test if such a phenomenon occurs in Argentine ants, we examined whether levels of intraspecific aggression vary as a function of queen presence or absence in experimental lab colonies. For each of four known supercolonies from southwestern California, we set up a pair of experimental colonies and randomly assigned replicates within each pair to treatment (queen removal) and control (no queen removal) groups. Using two different behavioral assays, we then measured aggressive behavior for ten days, removed queens from colonies in the treatment group, and continued to monitor aggression in both experimental groups for an additional 65 days. Both assays yielded qualitatively similar results: intraspecific aggression remained high throughout the experiment in both experimental groups. These results suggest that *L. humile* queens fail to influence levels of intraspecific aggression in introduced populations.

Key words: Argentine ants, intraspecific aggression, nestmate recognition, polygyny.

Introduction

Pheromones produced by social insect queens influence a diverse array of worker behaviors; this may be especially true for ants. Vienne et al. (1998), for example, found that in three

species of ants (*Manica rubida*, *Myrmica rubra*, and *Myrmica ruginodis*) groups of workers with queens exhibited a greater propensity for brood care compared to queenless groups, whereas workers in queenless groups were more likely to engage in nest protection. Furthermore, in the Argentine ant (*Linepithema humile*) variation in queen number influences attractiveness of queens to workers (Keller and Passera, 1989), and queen-primer pheromones control gyne production (Vargo and Passera, 1991) and the sex of brood eliminated by workers (Passera et al., 1995; Passera and Aron, 1996).

Queen pheromones can also influence nestmate-recognition behavior (Carlin and Hölldobler, 1986; Stuart, 1991; Starks et al., 1999). Queen-centered models of nestmate recognition involve systems in which queens synthesize chemical cues responsible for recognition odors, which are then distributed by workers (Lahav et al., 1998). Vander Meer and Alonso (2002), for example, recently documented an interesting case of queen-centered nestmate recognition in the red imported fire ant, *Solenopsis invicta*. They found that monogyne colonies lost intraspecific aggression within two weeks of experimental removal of queens and proposed that a queen-primer pheromone influences nestmate recognition in this species. Polygyne colonies of *S. invicta*, in contrast, exhibited low levels of intraspecific aggression both before and after queen removal (Vander Meer and Alonso, 2002).

In this study we test whether the experimental removal of queens influences intraspecific aggression in the Argentine ant (*L. humile*), a widespread invasive species (Suarez et al., 2001). Introduced populations of Argentine ants typically form expansive supercolonies within which intraspecific aggression is absent (Newell and Barber, 1913; Passera, 1994; Way et al., 1997; Tsutsui et al., 2000; Giraud et al., 2002). Both environmental (Liang and Silverman, 2000) and genetic (Tsutsui et al., 2000; Giraud et al., 2002) factors can contribute to variation in intraspecific aggression in this species, but little is known about the potential role of queens.

* Author for correspondence.

Although Argentine ants maintain highly polygynous colonies, several aspects of the ecology of this species contribute to create circumstances in which colony fragments could become temporarily queenless. These factors include the following: highly polydomous colony structure (Newell and Barber, 1913; Markin, 1968, 1970), seasonal execution of queens (Markin, 1970; Keller et al., 1989), and clustering of queens in certain nests (Aron, 2001). Given the potential for queenless colony fragments to occur in this species, it is of great interest to determine how queens might influence patterns of nestmate recognition. To investigate this phenomenon, we used lab colonies that were either queenright or had queens experimentally removed to test whether queens influence levels of intraspecific aggression between workers from mutually antagonistic supercolonies.

Methods

Collection and maintenance of lab colonies

We collected Argentine ants from four different sites in southwestern California: Los Peñasquitos Canyon Preserve, San Diego Co. (LP), Lake Hodges, San Diego Co. (LH), Sweetwater River (vic. Rancho San Diego), San Diego Co. (SR), and Lake Skinner, Riverside Co. (LS). These sites are each currently occupied by distinct supercolonies (Holway et al., 1998; Tsutsui et al., 2000, 2003; Suarez et al., 2002). Ants from LP belong to a supercolony that extends into northern California (Tsutsui et al., 2000); ants from the other three sites belong to more localized supercolonies (Suarez et al., 2002).

After collecting colony fragments from the field, we constructed experimental colonies each consisting of three queens, about 100 pieces of brood, and approximately 1000 workers. For each of the four collecting locations, we formed two experimental colonies. We housed each experimental colony in a circular plastic nesting container (28 cm in diameter) lined with Fluon™ to prevent ants from escaping. Inside each nesting container we placed five nest chambers (glass test tubes (16 mm X 150 mm) half-full of water and stopped with cotton). Nest chambers were covered with aluminum foil to keep them dark and were elevated approximately 5 mm above the floor of the nest container with wire stands. We reared experimental colonies under standardized environmental conditions (12:12 h light-dark cycle at 22°C) and fed them crickets and 20% sugar water every three days. One week after we established experimental colonies, we randomly assigned one colony from each location to a treatment group (queen removal) and a control group (no queen removal). Queen removals took place ten days after the start of behavioral assays (see below). We used an aspirator to remove queens from treatment colonies. After experimental colonies were assigned to experimental groups, but before queen removals were performed, we discovered that a single queen had died in each of three colonies: the LS queen-removal colony, the LP queen-removal colony, and the LH queenright colony. These queens were not replaced. We observed no additional queen mortality for the duration of the experiment.

Behavioral assays

To estimate levels of intraspecific aggression, we used two different behavioral assays. We modified the first method (Method 1) from an assay developed by Carlin and Hölldobler (1986); this method has been used previously with *L. humile* (Holway et al., 1998; Tsutsui et al., 2000; Suarez et al., 2002). We placed a pair of workers, one from each of two experimental colonies, together in a nine-dram plastic vial coated with Fluon™. Over a five-minute interval, we rated observed behaviors on a four-point scale: 1 = Touch (contacts that include prolonged anten-

tion), 2 = Avoid (contacts that resulted in one or both ants retreating rapidly in opposite directions), 3 = Aggression (a brief (i.e., \leq two s) expression of any of the following behaviors: lunging, biting, pulling of legs or antennae, apparent use of chemical defense compounds), and 4 = Fight (prolonged aggression between individuals usually resulting in death or dismemberment). We conducted five trials for each pairing of experimental colonies and used the mean of these trials in all analyses.

For Method 2, we adapted an approach used by Vander Meer and Alonso (2002). For a set of trials, we connected each nesting container to an empty foraging container (a container the same dimensions as a nesting container) via a wire bridge and placed approximately 1 ml of sugar water in the foraging container. Once 10–50 workers moved into the foraging container, we introduced a worker from a queenright stock colony originating from LP by allowing the worker to walk onto a strip of paper and then letting it walk off the paper and into the foraging container. To facilitate comparisons with Method 1 trials but unlike Vander Meer and Alonso (2002), we used the same four-point scale described above to quantify aggression between the introduced worker and workers from the experimental colony. For each experimental colony, we conducted five trials (separated by 10-min intervals) and used the mean of these trials in all analyses. Method 2 trials lasted up to five min; we aspirated workers engaging in level-4 aggression in order to avoid disturbing the other workers in the foraging container. In a few cases, we let Method 2 trials run for 10 min. These exceptions were due to low densities of workers present in the foraging container.

We measured intraspecific aggression every five days for the first 35 days of the experiment and then every ten days for the remaining 40 days. We conducted the following pairings for Method 1: LP – SR, LP – LS, LP – LH, SR – LH, SR – LS, and LS – LH. These pairings included: (1) workers from queenless colonies matched against workers from other queenless colonies, and (2) workers from queenright colonies matched against workers from other queenright colonies. Note that none of the Method 1 comparisons paired workers from queenless colonies against workers from queenright colonies. Method 2 pairings included LP – SR, LP – LS, and LP – LH. As a control, we also ran comparisons between LP – LP using Method 2. As with Method 1, we conducted one set of trials involving queenless colonies and another set with queenright colonies, but note that, unlike Method 1 trials, Method 2 trials involved colonies from both experimental groups encountering a lone worker from a queenright colony (from LP).

Although all of the pairings used for either Method 1 or Method 2 assays consisted of unique combinations of experimental colonies, because only four supercolonies are presently known from southern California (Suarez et al., 2002; Tsutsui et al., 2003), it was necessary to represent experimental colonies from particular sites more than once in different pairings. Our study shares this potential design limitation with the many published studies conducted on introduced populations of *L. humile* that treat samples collected from a single site (or from multiple sites in close proximity) as independent replicates. To the extent that our unique pairings are not truly independent of one another, the results of the parametric statistical tests run on these data should perhaps be interpreted with caution. To determine if levels of intraspecific aggression varied across time and as a function of the presence or absence of queens, we used repeated-measures analysis of variance with treatment group (queenright versus queenless) as the between-subjects variable and sampling period as the within-subjects variable. We ran separate ANOVAs for Method 1 and 2 assays.

Results

Removal of queens did not result in decreased levels of intraspecific aggression in experimental colonies of *L. humile* (Fig. 1). For both behavioral assays, levels of intraspecific aggression did not differ with respect to whether queens were present or absent: Method 1 (Fig. 1A; $F_{1,10} = 0.198$, $p > 0.05$) and Method 2 (Fig. 1B; $F_{1,4} = 0.893$,

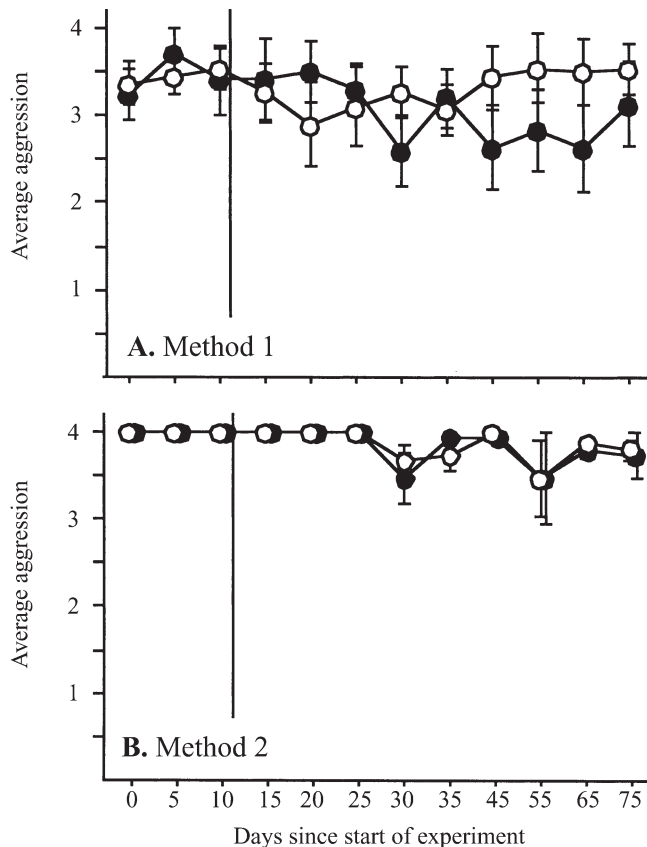


Figure 1. Mean (\pm SE) levels of intraspecific aggression for queenless (open circles) and queenright (closed circles) colonies of Argentine ants over a 75-day period. Intraspecific aggression estimated (A) for pairs of workers from different supercolonies (Method 1), and (B) for colonies that encounter a lone, foreign conspecific worker (from a queenright colony) while foraging (Method 2). The solid vertical line in both figures shows the timing of queen removal in the queenless treatment group. Each mean is the average response of each experimental group for a specific sampling date. Sample sizes for each mean are as follows: $n = 6$ for Method 1, $n = 3$ for Method 2

$p > 0.05$). Levels of intraspecific aggression also did not change over the course of the experiment: Method 1 (Fig. 1A; $F_{1,11} = 1.149$, $p > 0.05$) and Method 2 (Fig. 1B; $F_{1,5} = 0.822$, $p > 0.05$). Only the results for the last 45 days of the Method 2 experiment are included in the ANOVA because levels of intraspecific aggression exhibited no variation prior to this time point (Fig. 1B). In the trials conducted for the Method 2 control (LP-LP), we never observed any fighting; these results are not graphed.

Despite that the two behavioral assays used in this study differed in numerous respects (e.g., size of arena, presence of food, and number of ants), the two methods yielded qualitatively similar results. Differences between Method 1 and Method 2 scores were not significantly different from zero (one-sample t -tests: $p > 0.05$ for all comparisons) at any time point. Note that for these comparisons, we included only colony pairings common to both Method 1 and 2 (e.g., LP – SR, LP – LS, and LP – LH) but pooled the results of queenless and queenright colonies within each category of methods.

Discussion

Intraspecific aggression did not decrease with removal of queens from experimental colonies of Argentine ants (Fig. 1). Moreover, as with previous studies using these supercolonies (Holway et al., 1998; Suarez et al., 2002), levels of intraspecific aggression remained high throughout the experiment (Fig. 1). These findings suggest that Argentine ant queens do not emit pheromones that profoundly alter nestmate-recognition behavior, and that this species does not appear to conform to a queen-centered model of nestmate recognition.

These results seem consistent with several features of the ecology of *L. humile*. First, the tendency in Argentine ants for numerous queens to be distributed, often unevenly, among multiple, physically separate nests would seem to reduce the effectiveness of a queen-centered system of nestmate recognition. Moreover, individual nests that make up an Argentine ant colony are often ephemeral, and interchange of workers, queens, and brood among nests can be high (Newell and Barber, 1913; Markin, 1968, 1970). This within-colony flux of workers and queens might further compromise the effectiveness of queen-centered nestmate recognition.

The results of this study appeared insensitive to the type of behavioral assay used; both methods yielded qualitatively similar results (see also Roulston et al. (2003) for a detailed assessment of this issue). Method 1 could potentially provide a misleading impression of colony membership given that (1) workers are divorced from their social environment, (2) workers remain confined to small vials, and (3) workers become physically jostled during transport into the vials, which could artificially increase the likelihood of aggressive behavior. Method 2 lacks these shortcomings and emulates the more realistic scenario of a colony encountering a foreign worker while foraging. Nonetheless, the results of the Method 1 trials did not appear to differ from those of the Method 2 trials at any point in the experiment. Moreover, the results of both behavioral assays are consistent with previous observations (Holway et al., 1998) involving colony – colony interactions between the same supercolonies used here.

The results of this study suggest that nestmate recognition in *L. humile* does not conform to a queen-centered model (Lahav et al., 1998). Given that *L. humile* queens influence other aspects of colony dynamics (Vargo and Passera, 1991; Passera et al., 1995; Passera and Aron, 1996), however, it will be of interest to examine how queens might influence more subtle aspects of nestmate recognition than intraspecific aggression between non-nestmates as studied here. An additional caveat of our interpretation is that we focused exclusively on introduced populations. Native populations, which exhibit a higher frequency of intraspecific aggression and harbor more genetic variation (Tsutsui et al., 2000), might differ from introduced populations with respect to the relative importance of heritable versus environmentally-based nestmate-recognition cues. Given that introduced populations of most invasive ant species often display little to no intraspecific aggression across large spatial scales and given that the lack of intraspecific aggression may be an important

contributing factor in the success of these species in new environments, it will be of interest to develop a better understanding of the sources of variation in nestmate-recognition behavior in both native and introduced populations of these species.

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