

Fusion Between Southeastern United States Argentine Ant Colonies and Its Effect on Colony Size and Productivity

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ABSTRACT The ecological success of invasive ants has been linked to their ability to form expansive supercolonies. In the Argentine ant, *Linepithema humile* (Mayr), increased productivity and competitive ability of introduced supercolonies in several places, e.g., California and southern Europe, has been linked to high population densities that could have been attained via fusion of nonaggressive and genetically similar nests. Recently, we have found that introduced *L. humile* colonies in the southeastern United States, which have higher levels of intraspecific aggression and genetic diversity than those in California and southern Europe, sometimes also fuse; yet it is unclear what the longer term consequences of such colony fusion might be. In this study, we examined whether fusion of these southeastern United States *L. humile* colonies results in larger colonies by recording colony size and productivity in pairs that fused and in pairs that did not fuse. After 6 mo, colonies that fused produced 47% more workers and had twice as many queens as colony pairs that did not fuse. Also, fused colonies had an overall per capita colony productivity (number of brood and workers produced per queen and per worker) comparable to that of nonfused pairs and unpaired controls. Furthermore, all queens contributed to worker pupae production in fused colonies. Thus, fusion of initially aggressive southeastern United States *L. humile* colonies results in colonies with higher worker number without decreasing per capita productivity. Moreover, offspring contribution by all queens in fused colonies may alter colony genotypic composition resulting in reduced intraspecific aggression that in turn promotes further fusion. This process may be relevant to the establishment of incipient colonies in areas where multiple introductions have occurred.

KEY WORDS *Linepithema humile*, unicoloniality, aggression, colony fusion

Colony size plays a key role in determining social complexity, within-group conflict, colony productivity, behavioral flexibility, and colony organization in social insects (Karsai and Wenzel 1998, Bourke 1999). Typically, large colonies have improved defense, higher sexual production, and enhanced resistance to extreme climate (Adams 1990, Kaspari and Vargo 1995, Bourke 1999). In addition, individuals in social groups enlarged by incorporating unrelated members can enjoy fitness benefits (Costa and Ross 2003), which may extend to ecologically dominant unicolonial ant species (Hölldobler and Wilson 1977, Porter and Savignano 1990, Holway et al. 2002, Abbott 2005) for which ecological factors may contribute to a high benefit:cost ratio favoring altruism when relatedness is low (Foster et al. 2005).

The ecological success of invasive populations of the unicolonial Argentine ant, *Linepithema humile* (Mayr) has been linked to colony size, more specifically to high worker number (Holway 1999, Holway and Case 2001, Holway and Suarez 2004). It has been suggested that large worker numbers are possible because the lack of intraspecific aggression promotes investment in brood and worker production rather than colony defense (Holway et al. 1998). This loss of

aggression could be linked to low levels of genetic diversity, a consequence of a genetic bottleneck reducing phenotypic variability of nestmate recognition cues (Tsutsui et al. 2000). Alternatively, increased colony growth and size may result from fusion of colonies sharing the most common recognition alleles when populations are introduced into new habitats with relaxed ecological constraints (Giraud et al. 2002). Both of these explanations for increased colony size leading to supercolony (or unicolonial population) formation invoke a reduction in genetic diversity at nestmate recognition loci. However, recent evidence suggests that loss of genetic diversity may not be necessarily involved in the evolution of unicoloniality because native *L. humile* populations also form supercolonies but much smaller in size than the unicolonial populations in the introduced range (Pedersen et al. 2006). Thus, the formation of vast supercolonies in the introduced range may be linked to ecological conditions favoring changes in colony size.

Throughout its introduced range, the extent of Argentine ant unicoloniality and colony size varies (Tsutsui et al. 2000, Giraud et al. 2002, Buczkowski et al. 2004, Heller 2004), possibly owing to regional differences in genetic diversity, intraspecific aggression, and abiotic factors. The ecologically dominant super-

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colonies in California (Holway and Case 2001) may have formed via fusion of nonaggressive and genetically similar nests (Holway 1999). Unlike populations in California, *L. humile* colonies in the southeastern United States occupy relatively small territories, and display relatively high genotypic variability and intercolony aggression (Buczowski et al. 2004), yet they sometimes fuse depending on the degree of genetic and cuticular hydrocarbon profile similarity (Vásquez and Silverman 2008). Fusion of these spatially isolated and aggressive *L. humile* colonies may be a consequence of errors in nestmate recognition. Moreover, it is unclear whether fusion of aggressive colonies may result in larger colonies over the long-term. An increase in worker number via fusion may lead to larger and more productive colonies (Michener 1964, Oster and Wilson 1978) but with a possible reduction of per capita productivity associated with increased colony size (Michener 1964). Perhaps unrelated queens in fused colonies contribute equally to worker production as predicted from the low reproductive skew found among nestmate *L. humile* queens (Keller 1988, Fournier and Keller 2001), and this may explain, in part, reduced worker aggression through homogenization of colony recognition cues (Vásquez and Silverman 2008, Vásquez et al. 2009).

In this study we conducted a 6-mo-long experiment to investigate whether fusion of genetically distinct and geographically isolated *L. humile* colonies in the southeastern United States results in larger colonies with higher worker number than those that did not fuse. We compared the per capita production of colony members per queen and per worker between fused and nonfused pairs. We also investigated the reproductive contribution of queens in fused colonies. We reveal that fusion of genetically distinct *L. humile* colonies results in higher worker production without a decrease in per capita productivity, suggesting that fusion may contribute to the formation of larger colonies from originally mutually aggressive, smaller colony fragments.

Materials and Methods

Ant Colonies and Colony Fusion. Argentine ant colonies used in this study have been examined previously in a laboratory colony fusion study as described in Vásquez and Silverman (2008). Briefly, we established experimental *L. humile* colonies from five locations in the southeastern United States: Cary (CAR), Chapel Hill (CHH), Research Triangle Park (RTP), and Winston Salem (FOR), NC; and Greenville (COC), SC. Each experimental colony consisted of five queens, \approx 100 brood, and 500 workers. All queens and 50 workers from each colony were marked with water-based paint to observe individuals mixing and determine fusion events. Colony fusion was defined as the presence of surviving marked queens from both colonies and all brood in the same nest, and mixing of workers without fighting. In contrast, colonies that did not fuse were characterized by lack of cohabitation of marked queens and continuous aggression among workers of the two interacting colo-

Table 1. Initial levels of worker aggression and genetic similarity (% alleles shared and pairwise F_{ST}) for Argentine ant colony pairs examined in a 6-mo colony fusion assay

Colony pair	Fusion (%)	Aggression level	Alleles shared (%)	Pairwise F_{ST}
CAR-RTP	100	2.0	63.3	0.19
CHH-RTP	100	2.9	58.1	0.01
COC-FOR	100	3.4	62.1	0.21
CAR-FOR	40	4.0	54.8	0.20
FOR-RTP	40	3.5	60.6	0.25
CAR-CHH	20	4.0	51.7	0.21
CAR-COC	0	4.0	43.3	0.31
CHH-COC	0	4.0	30.3	0.48
CHH-FOR	0	3.3	62.5	0.29
COC-RTP	0	3.9	41.2	0.43

CAR, Cary; CHH, Chapel Hill; COC, Greenville; FOR, Winston Salem; RTP, Research Triangle Park.

nies, which generally resulted in elimination of all queens from one of the original colonies. After a 24-h acclimation period, colony pairs were connected and behavioral interactions were recorded as follows: total number of workers fighting and dead workers were recorded each hour for 6 h, and at 24 h after interactions between colonies started; colonies also were inspected for fusion for the first 6 h, at 24 h, daily for 30 d, and monthly, from month 2 to month 6. We tested all 10 pairwise combinations in three trials, with five colony pairs per trial and five replicates per pair. As reported previously (Vásquez and Silverman 2008), we found a correlation between worker aggression and colony fusion, and also between genetic similarity and colony fusion. Aggression levels (1–4 scale, low to high aggression) and genetic similarity (8 microsatellite loci) for colony pairs examined in this study are shown in Table 1. Moreover, colony pairs displaying lower intraspecific aggression and higher levels of genetic similarity had higher cuticular hydrocarbon similarity, which also was associated with higher fusion rates.

In this study, we examined whether colonies that fused in the previous study (Vásquez and Silverman 2008) differed in colony size and per capita productivity over time from those that did not; and we also investigated the contribution of queens to worker production in fused colony pairs. Unpaired colonies from each location were used as controls to determine if colony growth differed among all five colonies in the absence of intercolony interactions. As reported previously (Vásquez and Silverman 2008), fusion of the study colonies varied across colony pairs (Supp. Table 1 [online only]).

Total Colony Size and Per Capita Brood and Worker Production. We recorded the total number of workers, brood, and queens in each replicate at 24 h, and every month from month 1 to month 6. Data were analyzed as the total number of brood, workers, queens, and the proportion of queens surviving (number of queens alive per initial queen number) recorded monthly, and as per capita values (number of brood and workers per queen and per worker, respectively). Total numbers are useful to determine differences in colony size, whereas per capita values allow an easy comparison of colony growth rates and caste ratios among colonies with different sizes. Monthly

per capita values were obtained by dividing the number of brood by the number of workers (B/W) or queens (B/Q) recorded in the same month, the number of workers divided by the number of queens recorded in the same month (W/Q), and the number of workers recorded each month divided by total worker number recorded in the previous month (W/W).

Contribution of Queens to Offspring in Fused Colonies. We assessed the contribution of individual queens to offspring production in fused replicates of CAR-RTP, CHH-RTP, and CAR-CHH by genotyping queens and a sample of worker pupae taken 6 mo after the fusion assay started. Pupae collected at 6 mo are expected to be offspring of the queens present in the fused colony based on the 33–141 d it takes for eggs to develop into workers in this species (Newell and Barber 1913, Markin 1970). Genomic DNA was extracted from all queens (3–7) and 10 worker pupae per replicate (59 queens and 110 pupae total) using the DNeasy Tissue Kit (Qiagen, Valencia, CA) and analyzed at eight microsatellite loci: *Lhum-11*, *Lhum-13*, *Lhum-19*, *Lhum-28*, *Lhum-35*, *Lhum-39* (Krieger and Keller 1999), *Lihu-M1*, and *Lihu-T1* (Tsutsui et al. 2000). Polymerase chain reactions (PCR) were performed as described by Buczkowski et al. (2004). Products were separated on 6.5% KB^{Plus} polyacrylamide sequencing gels (Li-Cor Corp., Lincoln, NE) using a 4300 LI-COR DNA analyzer. Microsatellite alleles were scored using GeneImagIR software (Scanalytics Inc., Billerica, MA). For each replicate, we compared pupae genotypes with those of queens to examine the pedigree relationship between individual pupae and queens, and determine if observed genotype frequencies differed significantly from the expected under equal offspring production by queens. Also, we compared the allele composition of offspring in fused colonies with that of workers and queens sampled from each of the source colonies at the beginning of the fusion assay (Supp. Table 1 [online only]) to determine if colony-specific (private) alleles were present in offspring in fused colonies.

Statistical Analyses. All analyses were conducted using SAS 9.1.3 statistical software (SAS Institute 2004). Total number of workers, brood, and queens (square-root transformed) recorded on day 1 and 180 were compared between all replicates that fused and those that did not across all colony pairs and runs by using PROC MIXED with fusion (yes or no) and pair nested within fusion as fixed factors, and run by pair by fusion as a random interaction. Differences between replicates that fused and those that did not within the same colony pair were compared using PROC MIXED with fusion by pair as a fixed factor and run nested within pair as a random variable, followed by mean separation by LSMeans. Similar analyses were carried out for the proportion of brood and workers per queen (B/Q and W/Q) and per worker (B/W and W/W) estimated on days 1, 90, and 180. All means reported are followed by standard errors.

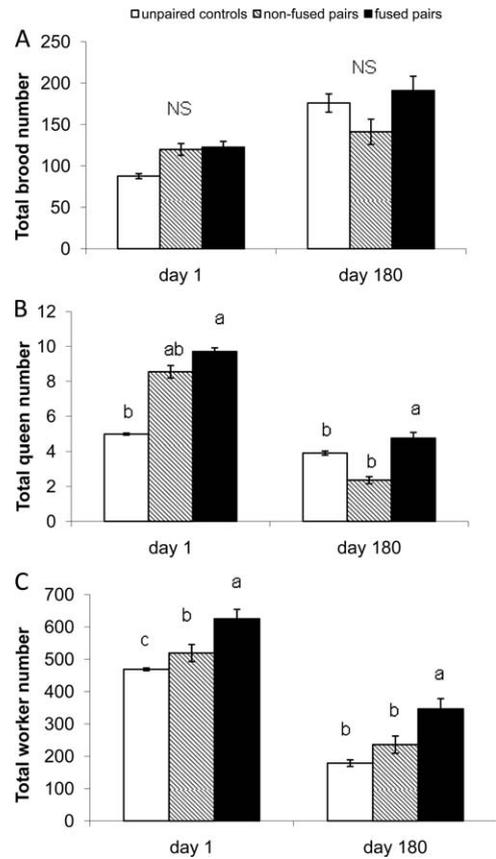


Fig. 1. Mean (± 1 SE) total brood (a) and worker (b) production and total queen number (c) for all colony pair replicates that fused versus those that did not and unpaired controls on days 1 and 180. Different letters indicate significant differences between means ($P < 0.05$, least significance difference test); NS, nonsignificant.

Results

Total Colony Size. In the analysis of fused and non-fused replicates averaged across colony pairs, we found that total brood number did not differ among all three groups (fused, nonfused and control) on day 1 ($F = 0.53$, $df = 2$, 16.7 , $P = 0.6010$) or day 180 ($F = 3.11$, $df = 2$, 18.5 , $P = 0.0683$) (Fig. 1a). Both the controls and the fused replicates increased brood production from day 1 to day 180, 101% and 56%, respectively ($t = 6.70$, $df = 118$, $P < 0.0001$, and $t = 3.25$, $df = 51$, $P = 0.0010$, respectively), unlike nonfused replicates that had equal numbers of brood at day 1 and on day 180 ($t = 1.15$, $df = 75$, $P = 0.1267$). Colony pairs and unpaired controls did not differ in number of brood produced ($F = 0.61$, $df = 14$, 14.8 , $P = 0.8181$) (Supp. Table 1 [online only]).

When we compared worker numbers between fused and nonfused replicates averaged across all colony pairs versus the control average, fused replicates had 1.2 and 1.3 times more workers than nonfused replicates and control, respectively, on day 1 ($F = 6.33$, $df = 2$, 20.9 , $P = 0.0071$), and 1.5 and 1.9 times more

on day 180 ($F = 6.38$, $df = 2,21.2$, $P = 0.0068$), respectively (Fig. 1b). Number of workers did not differ among unpaired controls (Supp. Table 1 [online only]). Fused replicates averaged across colony pairs had 1.1 and 1.9 times more queens than both nonfused replicates and the control average, respectively, on day 1 ($F = 89.04$, $df = 2,22.9$, $P < 0.0001$), and 2.0 and 1.2 times more queens than nonfused replicates and the control average, respectively, on day 180 ($F = 10.38$, $df = 2,19.9$, $P = 0.0008$) (Fig. 1c). As expected, queen survival was the highest in unpaired control versus fused and nonfused colonies ($F = 21.69$, $df = 14, 15$, $P < 0.0001$) (Supp. Table 1 [online only]).

Per Capita Brood and Worker Values. Fused and nonfused replicates averaged across colony pairs had B/Q values similar to the control average on day 1 ($F = 0.18$, $df = 2, 87$, $P = 0.8363$), day 90 ($F = 0.69$, $df = 2, 19.8$, $P = 0.5134$), and day 180 ($F = 0.25$, $df = 2, 21.1$, $P = 0.7793$) (Fig. 2a). Brood per queen (B/Q) differed among colony pairs ($F = 4.03$, $df = 14, 13$, $P = 0.0083$) (Supp. Table 2 [online only]).

Nonfused replicates averaged across colony pairs had higher B/W values than the control average on day 1 ($F = 4.92$, $df = 2, 8.1$, $P = 0.0399$), unlike day 90 where all three groups (fused, nonfused, and unpaired control) had similar B/W values ($F = 0.71$, $df = 2,19.1$, $P = 0.5060$) (Fig. 2b). On day 180, both fused and nonfused replicates had lower B/W values than the control average ($F = 6.00$, $df = 2, 18.9$, $P = 0.0096$) (Fig. 2b). Differences in proportion of brood per worker (B/W) between colony pairs and unpaired controls varied across sampling date ($F = 3.14$, $df = 84,737$, $P < 0.0001$) (Supp. Table 2 [online only]).

Fused and nonfused replicates averaged across colony pairs had lower W/Q values than the control average on day 1 ($F = 174.18$, $df = 2, 25.2$, $P < 0.0001$). In contrast, W/Q values were similar among fused and nonfused replicates and the control average on day 90 ($F = 0.40$, $df = 2, 22.3$, $P = 0.6738$), whereas W/Q was higher in fused and nonfused replicates than the control on day 180 ($F = 7.47$, $df = 2, 25.2$, $P = 0.0028$) (Fig. 2c).

Fused and nonfused replicates averaged across colony pairs had lower W/W than the control average on day 1 ($F = 262.40$, $df = 2, 22.7$, $P < 0.0001$), however, fused replicates had a higher W/W than nonfused replicates and control on day 90 ($F = 3.94$, $df = 2,21.2$, $P = 0.0350$), whereas no differences were found on day 180 ($F = 0.75$, $df = 2,17.4$, $P = 0.4875$) (Fig. 2d). Control colonies had a higher W/W than all colony pairs at 24 h ($F = 48.53$, $df = 14, 13$, $P < 0.001$) (Supp. Table 2 [online only]).

Contribution of Queens to Offspring in Fused Colonies. Maternal origin of worker pupae was determined by comparing queen and pupae genotypes at eight microsatellite loci as shown in Supp. Table 3 (online only) for one of the fused CHH-RTP replicates. There was no significant difference among queens in the number of worker pupae produced in fused replicates as determined by observed versus expected offspring genotype frequencies (highest $\chi^2 = 5.4 < \chi^2_{0.05,6} = 12.6$, Supp. Table 3 [online only]). In replicates where the identity of all queens was

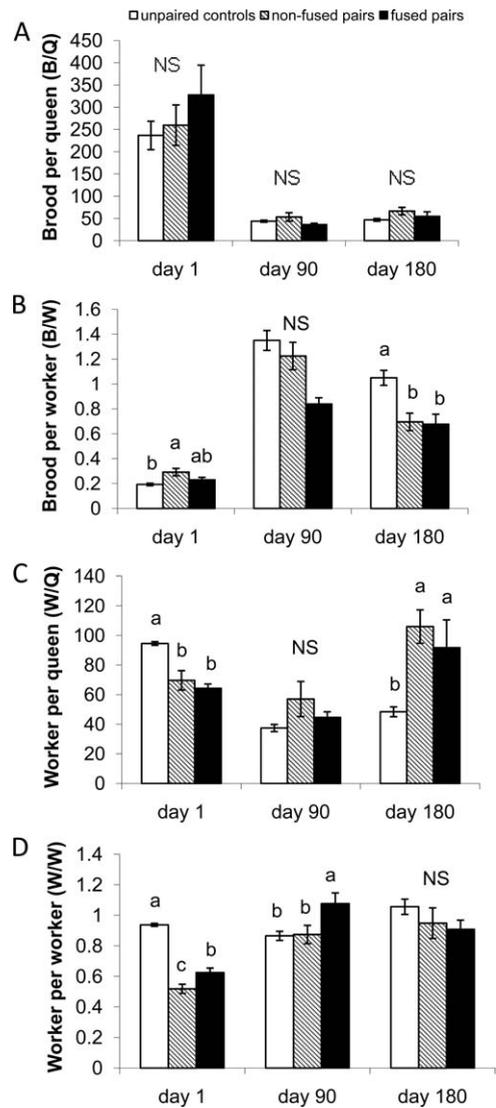


Fig. 2. Mean (± 1 SE) per capita brood production for queens (B/Q) (a) and for workers (B/W) (b) and mean (± 1 SE) per capita worker production for queens (W/Q) (c) and workers (W/W) (d) for all colony pair replicates that fused versus those that did not and unpaired controls on days 1, 90 and 180. Different letters indicate significant differences between means ($P < 0.05$, least significance difference test); NS, nonsignificant.

known, all worker pupae had genotypes consistent with those of the queens and in proportions expected based on the number of queens from each respective colony. For example, in one CAR-CHH replicate, 50% of the genotyped worker pupae were CHH and the other 50% were CAR, corresponding to two CHH and two CAR queens present. Similarly, in one CAR-RTP replicate with four CAR and three RTP queens, 60% of the worker pupae were CAR and 40% were RTP. Thus, the genotypic composition of worker offspring in mixed groups corresponded to the composition of

the queens present in those groups. The CAR:RTP, CHH:RTP, and CAR:CHH queen ratios at 6 mo were averaged (1.07:1) and found not to differ from the ratio expected in a mixed group with symmetrical queen composition (1:1) ($t = 0.1173$, $df = 5$, $P = 0.4556$); which also suggests a proportional worker pupae composition. In addition, fused colonies possessed not only alleles shared by both sources but also private alleles from each source colony (Supp. Tables 4 and 5 [online only]). Also, in a few fused replicates we observed alleles in pupae that did not match those of queens from either original colony (Supp. Table 5 [online only]) and thus may have had a paternal origin.

Discussion

We demonstrated that fusion of mutually aggressive *L. humile* colonies can result in colonies with increased worker production and higher queen number, and that all queens in these fused colonies contribute to worker production, which likely elevates within-colony genetic diversity leading to the formation of more open groups that may in turn increase their acceptance of individuals from other colonies. Unlike previous studies demonstrating that only nonaggressive colonies fused leading to larger brood and worker populations (Holway et al. 1998, Holway and Suarez 2004, Thomas et al. 2005), we have shown that aggressive *L. humile* colonies fused (Vásquez and Silverman 2008), and our results suggest that colony fusion is not only a by-product of inaccuracies in nestmate recognition but also can increase colony productivity. Argentine ants have been and will continue to be distributed widely through commerce (Suarez et al. 2001), making it likely that small fragments of unrelated colonies will occur in close proximity. We suggest that fusion between incipient *L. humile* colonies that do not necessarily share high levels of genetic similarity may result in larger and more productive colonies, and that such fusion events may occur during the early stages of invasive supercolony formation.

We showed previously (Vásquez and Silverman 2008) that colony pairs that fused were genetically more similar (51.7–63.3% alleles shared) than colony pairs that did not (30.3–62.50% alleles shared) even when pairs were formed from colonies originating up to 289 km apart, which indicates that fusion between aggressive colonies is regulated in part by genetic similarity. This conclusion is in line with studies showing that introduced supercolonies with high levels of genetic differentiation ($F_{ST} = 0.29$ – 0.54) do not fuse in the field (Jacquière et al. 2005, Thomas et al. 2006), and, moreover, these values are comparable to those of our colony pairs that did not fuse ($F_{ST} = 0.29$ – 0.47) (Vásquez and Silverman 2008). Variation in the likelihood and time of fusion between replicates of the same colony pair, suggest that factors such as differences in colony genetic composition (queen and worker genotypes), physiological and behavioral traits (queen reproductive status, worker age, proportion of aggressive worker phenotypes), or both may further regulate this process. In addition, differences in col-

ony phenology (age, reproductive stage, worker composition) also may influence the odds of winning intraspecific battles. Overall, certain intrinsic factors of colonies and their genotypic composition seem to be important in determining the outcome of intraspecific interactions between colonies.

Aggressive *L. humile* colonies that fused (replicates averaged across all colony pairs) had 1.5 times more workers and twice as many queens than aggressive colonies that did not fuse at the end of the experiment. In contrast, brood number between fused and nonfused colonies did not differ. Holway et al. (1998) reported greater differences in worker and brood numbers between nonaggressive (fused) and aggressive (nonfused) colonies than in this study. Higher initial worker mortality and lower worker numbers in our aggressive pairs that ultimately fused may have affected brood production (Oster and Wilson 1978). In addition, variation in colony genetic and phenotypic composition and other intrinsic colony traits (queen age and reproductive status, age of worker) could explain the variation in total brood and worker numbers among fused and nonfused aggressive pairs.

In social insects, as colonies grow per capita productivity decreases (Michener 1964) following the law of diminishing returns. Interestingly, *L. humile* colonies that fused generally did not have lower per capita brood production than unpaired and smaller control colonies. Number of brood per queen (B/Q) did not differ between control and fused and nonfused replicates, and was within the range reported for nests in introduced *L. humile* populations (Ingram 2002). Number of brood per worker (B/W) also was within the range found in natural conditions (Markin 1970). Our results differ from a previous study where an inverse relationship between per capita brood production and colony size was observed in small *L. humile* propagules (10–1,000 workers) (Hee et al. 2000), however, per capita brood production was unrelated to worker number in larger colonies (>1,000 workers) in the same study. Interestingly, the only per capita value that differed between fused and nonfused replicates was number of workers per worker (W/W), with fused replicates having a higher rate of new worker production than the smaller, nonfused replicates on day 1 and day 90, which is in line with previous findings where per capita worker productivity increased with colony size in *L. humile* laboratory colonies (Rosset et al. 2005).

Direct fitness benefits of merging between unrelated social groups (e.g., improved task performance or increased disease resistance) may arise from increased genotypic diversity or from increased group size regardless of genetic variation if it improves resource exploitation, defensive behaviors, or overall colony performance (Oster and Wilson 1978, Herbers 1984, Sudd and Franks 1987, Herbers 1993, Costa and Ross 2003). For example, merging of incipient *Solenopsis invicta* Buren nests through brood raiding results in very large dominant nests (Tschinkel 2006) and large *L. humile* colonies have greater short-term task efficiency (food collection, territory exploration) and colony productivity (worker larvae and sexual

production) than small ones (Rosset et al. 2005). We found that fused colonies produced more workers, which could translate into more successful colonies because larger groups of *L. humile* workers retrieve more food, are better in competitive exploitation, and have a greater ability to monopolize resources (Human and Gordon 1996, Holway and Case 2001, Holway and Suarez 2004). In *S. invicta*, worker numbers determine brood production, colony growth, and survival in incipient colonies (Tschinkel 2006), whereas larger colonies produce more sexuals earlier and can undergo greater worker losses and still recover (Vargo 1988). Although not examined here, increased sexual production also may occur in fused *L. humile* colonies because high worker numbers can lead to higher rates of sexual production (Michener 1964).

In ants, variation in reproduction among nestmate queens is extensive, with queens sharing reproduction relatively equally in some species, where in others a single queen can monopolize reproduction (Keller and Vargo 1993). We found that non-nestmate queens in colony pairs that had fused contributed to production of worker pupae, consistent with the truly polygynous nature of this species (Keller 1988, Fournier and Keller 2001) and suggesting minimal within-colony conflicts for worker production. Whether reproductive skew for sexual production, and therefore queen direct fitness, is high in these unrelated fused colonies remains to be determined, although it seems unlikely as suggested by the low reproductive skew reported previously between nestmate queens in *L. humile* (Fournier and Keller 2001). Workers in the newly fused colony possessed alleles from both original colonies, thus, we suggest that colony fusion may lead to changes in colony genetic structure, and perhaps more behavioral specialization and greater ergonomic efficiency (Moritz and Page 1999) although high genetic diversity may not necessarily lead to greater specialization (Rosset et al. 2005). Moreover, these changes may result in a broader recognition template through an increase in phenotypic variability of genetically-derived recognition cues, thereby forming more open colonies that may accept individuals from other unrelated colonies.

The dynamics of colony fusion and its effects on colony productivity may be most relevant to incipient *L. humile* colonies or small fragments dispersing by colony budding, rather than larger colonies (Newell and Barber 1913, Ingram 2002). Also, our experimental colonies may not reflect field colony worker composition because aggressive encounters could have eliminated older workers, more aggressive workers, or both, leaving only nurses and less efficient foragers, which may not occur in larger, field colonies. However, higher growth rates and increased productivity than would occur in the field could result from ad libitum feeding and controlled laboratory conditions. Nevertheless, our results clearly reflect the role of intrinsic colony traits in regulating intraspecific interactions. Growth rates and productivity between small and large ant colonies may differ, because small colonies invest mostly in workers to grow rapidly, whereas large colonies alternate investing in reproductives and workers (Oster and Wilson 1978). Therefore,

we suggest that studies examining fusion between larger colonies or colonies of a size that yields the greatest colony productivity, i.e., worker or pupae produced per queen (Sudd and Franks 1987), would shed light on alternative competitive strategies and possible fitness consequences. We investigated intrinsic factors that may be regulating colony fusion; however, ecological factors also must be important determinants of fusion in *L. humile*. Thus, how environmental (nesting site, temperature variation, humidity) and biotic factors (interspecific competitors, food availability) affect this process warrants further investigation.

Little to no gene flow between established introduced *L. humile* supercolonies suggests that fusion between aggressive colonies is unlikely (Jacquiéry et al. 2005, Thomas et al. 2006) and more recent studies indicate that supercolonies may not have originated by fusion (Corin et al. 2007, Vogel et al. 2010); however, we cannot rule out the possibility that two or more moderately genetically similar colonies could fuse into larger colonies that only accept individuals that bear similar recognition cues during the early stages of supercolony formation. Our results indicate that fusion of initially aggressive colonies can lead to the formation of larger colonies with an altered genetic structure but without a loss in per capita productivity. We suggest that fusion of *L. humile* colonies with intermediate to relatively high levels of genetic similarity is a possible mechanism involved in the establishment of incipient colonies in areas of multiple *L. humile* introductions.

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