

Cooperative colony founding alters the outcome of interspecific competition between Amazonian plant-ants

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Abstract In myrmecophytes, plants with structures in which ants establish colonies, there is strong competition among ant queens for access to host plants. However, our knowledge of how queens of different partner species interact when attempting to colonize plants remains limited. The Amazonian myrmecophyte *Maieta guianensis* is colonized by queens of two ant species: *Crematogaster laevis* and *Pheidole minutula*. We elucidated the competitive ranking of queens of these species and tested the hypothesis that cooperative colony founding (pleometrosis) by *P. minutula* queens could alter this ranking. We found

that *C. laevis* queens are behaviorally dominant to *P. minutula* when individual queens encounter each other. Despite being inferior in combat, however, *P. minutula* queens successfully colonized seedlings at similar rates whether they were placed alone or in concert with a *C. laevis* queen. This may have occurred because the smaller *P. minutula* queens frequently entered domatia before the more robust *C. laevis* queens. Although *C. laevis* queens can evict *P. minutula* queens that had previously colonized domatia, this was an infrequent phenomenon—perhaps because while not fatal, conflicts often resulted in serious injury. Furthermore, by colonizing the same plant cooperative *P. minutula* queens dramatically reduce the probability that *C. laevis* colonizes host-plants without reducing their own per capita rates of colonization success. To our knowledge, this is a novel benefit of pleometrosis, whose primary advantages have primarily been thought to occur after the critical stage of colony establishment. Given the decreased likelihood of colonization when faced with multiple *P. minutula*, it may be that *C. laevis*' persistence at the landscape level is enhanced by such factors as priority effects, superior dispersal ability, or niche partitioning.

Keywords *Crematogaster laevis* · *Maieta guianensis* · Myrmecophytes · *Pheidole minutula* · Pleometrosis

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Introduction

Myrmecophytic plants, those in which specialized ants establish colonies in hollow thorns, foliar pouches, or other domatia and defend host-plants against herbivores, are model systems with which to study interspecific mutualisms (reviewed in Bronstein, 1998; Heil and McKey,

2003). However, ant–plant systems are also exceptional ones with which to investigate interspecific competition for temporally or spatially fluctuating resources. This is because there is a strong competition among specialist ant queens utilizing the same species of host-plant for access to domatia to establish colonies (Davidson et al., 1989; Yu et al., 2004). In addition, while small plants are often simultaneously colonized by multiple queens, in most systems mature plants typically house only a single ant colony (Davidson et al., 1989; Bruna et al., 2005; but see Trager and Bruna, 2006).

In some ant species, queens cooperate with each other to find colonies. This behavior, known as pleometrosis, is thought to be advantageous over single-queen colony founding (haplometrosis) because cooperatively founded colonies have higher survival rates, increased rates of growth, and elevated success in territorial fights and brood raids (reviewed in Hölldobler and Wilson, 1990). In plants, pleometrosis may also provide an advantage against other species using the same species of host-plant (Vasconcelos, 1993; Feldhaar et al., 2005), especially if there are strong asymmetries in competitive ability (e.g., Palmer, 2004).

Maieta guianensis (Melastomataceae) is an understory myrmecophyte that forms mutualistic interactions with the ants *Crematogaster laevis* and *Pheidole minutula* (hereafter *Crematogaster* and *Pheidole*). Both ant species establish colonies in paired pouches located at the base of leaves; these domatia occur as two chambers with independent entrances on either side of a leaf's midrib (Nery and Vasconcelos, 2003). However, field surveys have demonstrated that the majority of *M. guianensis* plants are colonized by *Pheidole* (Vasconcelos and Davidson, 2000), and that established plants are inhabited by a mature colony of only one ant species (Vasconcelos, 1993). The mechanisms underlying *Pheidole*'s numerical dominance are unclear. Because there are very few vacant plants to establish colonies (only 6.6–12.1% of >600 plants in different habitats; Vasconcelos et al., unpubl.), and because there is limited turnover of colonies following their establishment (Vasconcelos and Davidson, 2000), it is likely that events occurring at the colony-founding stage have a large impact on these patterns. In particular, it has been hypothesized that cooperative colony founding by *Pheidole* queens (Vasconcelos, 1993) gives them a competitive advantage over those of *Crematogaster*, despite the putative advantage *Crematogaster* queens have in direct conflict resulting from their much larger body sizes (Nery and Vasconcelos, 2003).

Here, we present the results of a series of experiments conducted to elucidate the role of competition between queens of different plant-ant species for access to domatia, and in particular the role played by cooperative colony

founding. In our experiments we (1) establish the competitive ranking between *Crematogaster* and *Pheidole* queens and (2) evaluate if cooperative behavior among founding *Pheidole* queens could alter the structure of the competitive ranking determined by aggression. If the founding success of these species is altered by the number of *Pheidole* queens, this could influence the competitive hierarchy of these species and help explain the numerical dominance of *Pheidole* in this system.

Materials and methods

Study site and collection of ant queens

This study was conducted at the Biological Dynamics of Forest Fragments Project, located ca. 70 km north of Manaus, Brazil (2°30'S, 60°W). All experiments were conducted between July 2006 and August 2007 in a shade-house at Reserve #1501, an 800 ha expanse of non-flooded lowland forest (for a complete description of the site see Bierregaard et al., 2002). We conducted all experiments using dealate queens. To collect queens we opened the domatia of *Maieta guianensis* seedlings and removed any queens that had not yet produced workers. Queens were then isolated in plastic tubes for 24–48 h prior to their use in the experiments. Any queens that suffered injury during collection were not used in the experiment.

Establishing dominance hierarchies

To determine the outcome of direct contact between the *Crematogaster* and *Pheidole*, we simultaneously placed one queen of each species 3 cm apart from each other in a Petri dish ($N = 23$ trials). We then observed the queens for 25 min and recorded any evidence and consequences of interspecific aggression between queens. At the end of each trial we recorded whether a queen had died or sustained injury (e.g., lost legs or antennal segments, showed difficulty moving). Each queen was used for only one experimental trial. We then tested the hypotheses (1) that the frequency of conflicts between queens was non-random, (2) that queens of each species were equally likely to initiate attacks, and (3) that *Pheidole* queens were equally likely to fight or flee following attacks by *Crematogaster*. Hypotheses 1 and 3 were tested with Chi-squared tests, while Hypothesis 2 was tested with a binomial test because the Chi-squared test cannot be used in cases where there are no observations of one possible outcome. Finally, we used a G test to compare how the frequency of queens of each species that are dead/injured following attacks varied as a function of a *Pheidole* queen's behavioral response to attacks by a *Crematogaster*.

Effect of queen number on colonization success

We then conducted an experiment to determine how the presence of heterospecific queens influences the probability of colonizing host-plants. The seedlings used in these experiments were cultivated from seeds collected in our study site; *M. guianensis* seedlings were grown until they produced a single domatia-bearing leaf. Because each chamber of the leaf pouch has its own entrance, it is possible for the two domatia on each leaf to be colonized independently.

In the first treatment we investigated colonization behavior in the absence of competition by placing a single queen at the centre of the domatia-bearing leaf's upper surface ($N = 16$ trials for each species). In the second treatment we simultaneously placed a single queen of each species on the leaf ($N = 16$ trials). In the third treatment one *Crematogaster* queen was placed on the leaf with three *Pheidole* queens ($N = 16$ trials). We recorded a "colonization success" when an individual queen entered into a leaf pouch's chamber and remained there for 24 h. We used G tests to compare the frequency of colonization by *Pheidole* and *Crematogaster* in the different experimental treatments.

Results

Queens entered in conflict in 16 of the 23 experimental trials (69.5%; $\chi^2 = 5.3$, $df = 1$, $P = 0.02$). In the 16 trials where there were antagonistic interactions between queens, all were initiated by *Crematogaster* (Sign test $P < 0.0001$;

Fig. 1). Once *Crematogaster* attacked, *Pheidole* was more likely to counterattack than flee (counterattacks in 12 of 16 trials, $\chi^2 = 4$, $df = 1$, $P = 0.045$; Fig. 1). No *Crematogaster* queens died following conflicts, although 7 of 16 were injured. In contrast, all 16 *Pheidole* queens were died or injured as a result of conflicts with *Crematogaster* ($G = 16.1$, $df = 1$, $P < 0.0001$; Fig. 1).

In the second experiment, *Pheidole* queens successfully colonized seedlings at similar rates whether they were placed alone or in concert with a heterospecific queen (75 vs. 68.8%, respectively; $G = 1.55$, $df = 1$, $P = 0.69$; Table 1). There was also no difference in per-capita colonization rate when comparing solitary *Pheidole* queens with three *Pheidole* queens placed on a seedling along with a *Crematogaster* queen ($G = 0.23$, $df = 1$, $P = 0.63$). All of the plants in these trials were colonized, and 81.25% of them were colonized by more than one *Pheidole* queen ($N = 9$ by two queens and $N = 4$ by three queens). In 7 out of 9 trials when two *Pheidole* queens colonized the plant, they occupied the same leaf pouch, whereas in the remaining two trials they occupied different pouches of the same leaf. When three queens colonized the plant, the three occupied either the same pouch ($N = 2$) or the two pouches of the same leaf ($N = 2$, with one queen in one pouch and two in the other).

The colonization success of solitary *Crematogaster* queens was similar to that of those competing with a single *Pheidole* ($G = 0.5$, $df = 1$, $P = 0.48$), despite the fact that *Pheidole* queens entered domatia more quickly than *Crematogaster* queens (13.6 min \pm 13 SD vs. 42.3 min \pm 30 SD; Mann-Whitney $U = 63.5$, $P = 0.02$). When *Crematogaster* queens were competing for domatia with

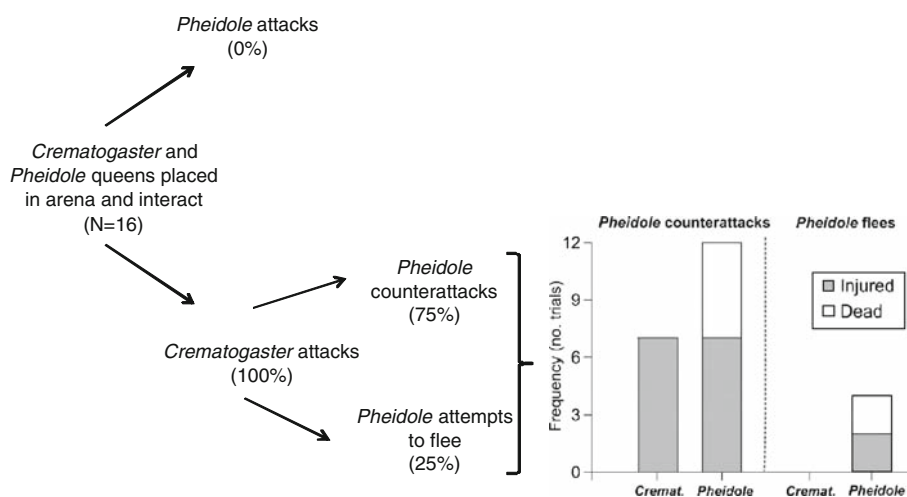


Fig. 1 Flow chart describing the outcome of the 16 trials in which there was interaction when single queens of *Pheidole minutula* and *Crematogaster laevis* were simultaneously placed in an area and observed for 25 min ($N = 23$ trials total). Percentages indicate how

often a behavior was observed in the 16 trials; the histogram shows the frequency of injury or death to queens of each species as a function of whether or not a *Pheidole* queen responds aggressively when attacked by *Crematogaster*

Table 1 *Per capita* colonization rates of *Maieta guianensis* seedlings by queens of *Crematogaster laevis* and *Pheidole minutula* and the number of colonized seedlings by each ant species

Treatment (<i>N</i> = 16 trials for each treatment)	<i>Per capita</i> colonization rate of <i>Pheidole</i> queens (%)	<i>Per capita</i> colonization rate of <i>Crematogaster</i> queens (%)	Seedlings colonized by <i>Pheidole</i> (%)	Seedlings colonized by <i>Crematogaster</i> (%)	Seedlings colonized by both species (%)
One <i>Pheidole</i>	75.00	–	75.00	–	–
One <i>Crematogaster</i>	–	56.25	–	56.25	–
One <i>Pheidole</i> + one <i>Crematogaster</i>	68.75	43.75	56.25	31.25	12.50
Three <i>Pheidole</i> + one <i>Crematogaster</i>	68.75	6.25	93.75	0.00	6.25

Values represent the percent of the total number of trials. In the *per capita* values, the numbers do not always sum to 100% because in three trials the queens of both species persisted on the plant for more than 24 h

three *Pheidole* queens, however, their success decreased sevenfold ($G = 10.34$, $df = 1$, $P = 0.001$; Table 1). Simultaneous colonizations by the two ant species occurred in only three of the 32 trials. In these cases each species was found in a different leaf pouch. *Crematogaster* attempted to displace established *Pheidole* queens in four of the 32 trials conducted with queens of both species (two from domatia with single queens and two from domatia with multiple queens). However, it only succeeded in the two attempts against single *Pheidole* queens—in the remaining two cases it either died or fled. *Pheidole* queens never attempted to displace *Crematogaster* queens.

Discussion

Ants that defend plants from herbivores in exchange for rewards such as food or shelter are one of the defining characteristics of tropical forests with over 200 species of myrmecophytic plants in Amazonia alone (Benson, 1985). Following the pioneering work of Janzen (1966; 1967), a number of studies have experimentally confirmed that ants can reduce rates of herbivory to host plants (reviewed in Bronstein, 1998; Rico-Gray and Oliveira, 2007) as well as influence plant fitness (e.g., Vasconcelos, 1991; Yu and Pierce, 1998; Izzo and Vasconcelos, 2002). Although numerous studies have investigated the factors influencing the successful colonization of plants by queens (Vasconcelos, 1993; Yu and Davidson, 1997; Frederickson, 2006), our knowledge of how queens of different partner species interact when attempting to colonize plants remains limited.

Our first experiment indicated that *Crematogaster* queens are behaviorally dominant to those of *Pheidole*. Antagonistic interactions during these trials were always initiated by *Crematogaster* queens, which killed nearly half of their opponents while suffering no fatalities. However, despite being inferior in combat, *Pheidole* queens successfully colonized seedlings at similar rates whether they

were placed alone or in concert with a *Crematogaster* queen. This may have occurred because battles between heterospecific queens were much less frequent on *Maieta guianensis* seedlings (7 of 32 trials; 21.9%) than they were in our experimental arenas (16 of 23 trials; 69.6%). Furthermore, the smaller *Pheidole* queens frequently entered domatia before the more robust *Crematogaster* ones, which sometimes had to enlarge the entrance to the leaf pouch (Davidson et al. 1989, Vasconcelos 1993). Although in our trials we observed *Crematogaster* queens evicting *Pheidole* queens that had previously colonized domatia, this was an infrequent phenomenon. This may be because while not fatal, conflicts with *Pheidole* often resulted in serious injury (Fig. 1).

Crematogaster queens successfully colonized seedlings at similar rates whether they were placed alone or in concert with a single *Pheidole* queen. In the presence of multiple *Pheidole*, however, the rate of successful seedling colonization by *Crematogaster* queens declined sharply. This decline was not driven by increased rates of *Crematogaster* mortality stemming from conflicts with cooperative *Pheidole*, although one *Crematogaster* queen was killed trying to invade a previously colonized leaf pouch with multiple *Pheidole* queens. Rather, colonization success was low because *Crematogaster* queens simply abandoned host plants with multiple *Pheidole*. This is somewhat surprising, given the rarity of and the difficulty in locating host-plants (Fonseca, 1999; Frederickson, 2006). We suggest that the abandoning of plants by *Crematogaster* may have occurred as a result of the pre-emption of domatia by *Pheidole*, which entered domatia in less than half the time than *Crematogaster* queens. *Crematogaster* spent an extended period of time antennating the external surface and the entrance of the leaf pouches, probably as means of verifying if the plant was already colonized and, if so, by how many queens. This could potentially explain why we observed few simultaneous colonizations, the occasional expulsion of solitary *Pheidole* queens by *Crematogaster*, and *Crematogaster* queens

abandoning plants despite the availability of, in most cases, one remaining empty leaf pouch.

Strictly speaking, cooperative colony founding by *Pheidole* only begins when multiple queens join each other inside the leaf pouch. However, our data indicate that the presence of multiple queens on the plant surface is also beneficial to individual queens. By colonizing the same plant, cooperative *Pheidole* queens dramatically reduce the probability that *Crematogaster* successfully colonizes host-plants without reducing their own per capita rates of colonization success. To our knowledge, this is a novel benefit of pleometrosis, whose primary advantages have primarily been thought to occur after the critical stage of colony establishment (Tschinkel and Howard, 1983). It is possible that the likelihood of queens fleeing plants on which there are multiple competitor queens may be influenced by factors not manipulated (e.g., host-plant quality, number of prior colonization attempts, seasonality), and our experiment was not designed to evaluate the fitness consequences of these interactions for either ant colonies or plants. However, they nevertheless suggest that behavioral mechanisms can influence competition for plants in which to establish colonies in ways that have previously remained unexplored (see also Dáttilo et al., 2009). Given the decreased likelihood of colonization when faced with multiple *Pheidole*, it may be that *Crematogaster*'s persistence at the landscape level is enhanced by priority effects (Vasconcelos, 1993), superior dispersal ability, or niche partitioning (reviewed in Palmer et al., 2002). Coupled with ongoing experimental, observational, and theoretical studies, our results are an important first step toward better elucidating the factors that promote the co-existence of multiple ant-species in this community of mutualists.

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