

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

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8 **Abstract** In myrmecophytes, plants with structures in
9 which ants establish colonies, there is strong competition
10 among ant queens for access to host plants. However, our
11 knowledge of how queens of different partner species
12 interact when attempting to colonize plants remains lim-
13 ited. The Amazonian myrmecophyte *Maieta guianensis* is
14 colonized by queens of two ant species: *Crematogaster*
15 *laevis* and *Pheidole minutula*. We elucidated the competi-
16 tive ranking of queens of these species and tested the
17 hypothesis that cooperative colony founding (pleometrosis)
18 by *Pheidole* queens could alter this ranking. We found that

C. laevis queens are behaviorally dominant to *Pheidole* 19
when individual queens encounter each other. Despite 20
being inferior in combat, however, *P. minutula* queens 21
successfully colonized seedlings at similar rates whether 22
they were placed alone or in concert with a *Crematogaster* 23
queen. This may have occurred because the smaller 24
P. minutula queens frequently entered domatia before the 25
more robust *C. laevis* queens. Although *C. laevis* queens 26
can evict *P. minutula* queens that had previously colonized 27
domatia, this was an infrequent phenomenon—perhaps 28
because while not fatal, conflicts often resulted in serious 29
injury. Furthermore, by colonizing the same plant cooper- 30
ative *P. minutula* queens dramatically reduce the 31
probability that *C. laevis* colonizes host-plants without 32
reducing their own per capita rates of colonization success. 33
To our knowledge, this is a novel benefit of pleometrosis, 34
whose primary advantages have primarily been thought to 35
occur after the critical stage of colony establishment. Given 36
the decreased likelihood of colonization when faced with 37
multiple *P. minutula*, it may be that *C. laevis*' persistence 38
at the landscape level is enhanced by such factors as pri- 39
ority effects, superior dispersal ability, or niche 40
partitioning. 41
42

Keywords *Crematogaster laevis* · *Maieta guianensis* · 43
Myrmecophytes · *Pheidole minutula* · Pleometrosis 44
45

Introduction 46

Myrmecophytic plants, those in which specialized ants 47
establish colonies in hollow thorns, foliar pouches, or other 48
domatia and defend host-plants against herbivores, are 49
model systems with which to study interspecific mutual- 50
isms (reviewed in Bronstein, 1998; Heil and McKey, 51

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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 ant–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., unpublished), 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 *P. minutula* 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 *C. laevis* and *P. minutula*, 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*.



152 Effect of queen number on colonization success

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

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

175 **Results**

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

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

187 In the second experiment, *P. minutula* queens success-
188 fully colonized seedlings at similar rates whether they were
189 placed alone or in concert with a heterospecific queen (75
190 vs. 68.8%, respectively; $G = 1.55$, $df = 1$, $P = 0.69$;
191 Table 1). There was also no difference in per-capita coloni-
192 zation rate when comparing solitary *Pheidole* queens
193 with three *Pheidole* queens placed on a seedling along with
194 a *C. laevis* queen ($G = 0.23$, $df = 1$, $P = 0.63$). All of the
195 plants in these trials were colonized, and 81.25% of them
196 were colonized by more than one *Pheidole* queen ($N = 9$
197 by two queens and $N = 4$ by three queens). In 7 out of 9
198 trials when two *Pheidole* queens colonized the plant, they
199 occupied the same leaf pouch, whereas in the remaining
200 two trials they occupied different pouches of the same leaf.
201 When three queens colonized the plant, the three occupied
202 either the same pouch ($N = 2$) or the two pouches of the
203 same leaf ($N = 2$, with one queen in one pouch and two in
204 the other).

205 The colonization success of solitary *C. laevis* queens
206 was similar to that of those competing with a single
207 *P. minutula* ($G = 0.5$, $df = 1$, $P = 0.48$), despite the fact
208 that *Pheidole* queens entered domatia more quickly than
209 *Crematogaster* queens (13.6 min \pm 13 SD vs. 42.3 min \pm
210 30 SD; Mann-Whitney $U = 63.5$, $P = 0.02$). When
211 *Crematogaster* queens were competing for domatia with

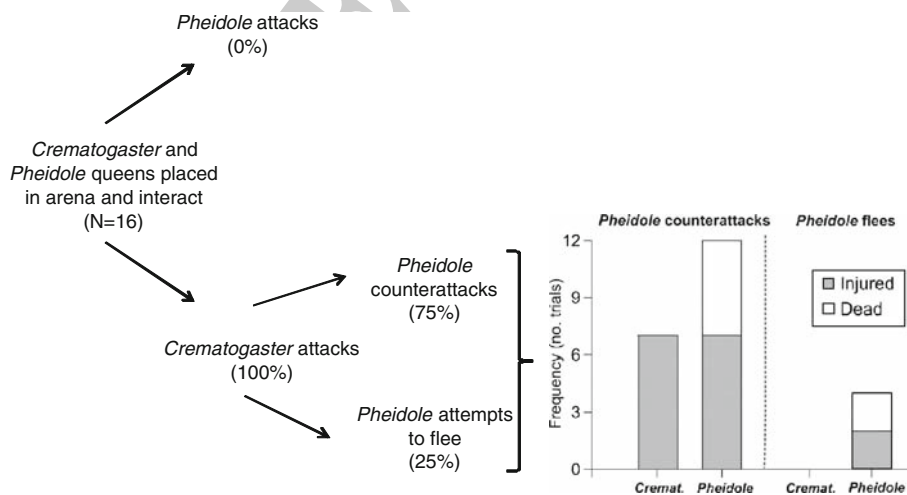


Fig. 1 Flow chart describing the outcome of the 16 trials in which there was interaction when single queens of *Pheidole minutula* and *Crematogaster laevis* were simultaneously placed in an arena and observed for 25 min ($N = 23$ trials total). Percentages indicate how often a behavior was observed 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 (N = 16 trials for each treatment)	Per capita colonization rate of <i>Pheidole</i> queens (%)	Per capita 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

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

224 Discussion

225 Ants that defend plants from herbivores in exchange for
 226 rewards such as food or shelter are one of the defining
 227 characteristics of tropical forests with over 200 species of
 228 myrmecophytic plants in Amazonia alone (Benson, 1985).
 229 Following the pioneering work of Janzen (1966; 1967), a
 230 number of studies have experimentally confirmed that ants
 231 can reduce rates of herbivory to host plants (reviewed in
 232 Bronstein, 1998; Rico-Gray and Oliveira, 2007) as well as
 233 influence plant fitness (e.g., Vasconcelos, 1991; Yu and
 234 Pierce, 1998; Izzo and Vasconcelos, 2002). Although
 235 numerous studies have investigated the factors influencing
 236 the successful colonization of plants by queens
 237 (Vasconcelos, 1993; Yu and Davidson, 1997; Frederickson,
 238 2006), our knowledge of how queens of different partner
 239 species interact when attempting to colonize plants remains
 240 limited.

241 Our first experiment indicated that *C. laevis* queens are
 242 behaviorally dominant to those of *P. minutula*. Antagonistic
 243 interactions during these trials were always initiated by
 244 *Crematogaster* queens, which killed nearly half of their
 245 opponents while suffering no fatalities. However, despite
 246 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 phe-
 nomenon. This may be because while not fatal, conflicts with
Pheidole often resulted in serious injury (Fig. 1).

C. laevis queens successfully colonized seedlings at
 similar rates whether they were placed alone or in concert
 with a single *P. minutula* queen. In the presence of multiple
Pheidole, however, the rate of successful seedling coloni-
 zation 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

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

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

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