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A temporary social parasite of tropical plant-ants improves the fitness of a myrmecophyte

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Abstract Myrmecophytes offer plant-ants a nesting place
in exchange for protection from their enemies, particularly
defoliators. These obligate ant–plant mutualisms are com-
mon model systems for studying factors that allow
horizontally transmitted mutualisms to persist since para-
sites of ant–myrmecophyte mutualisms exploit the rewards
provided by host plants whilst providing no protection in
return. In pioneer formations in French Guiana, *Azteca
alfari* and *Azteca ovaticeps* are known to be mutualists of
myrmecophytic *Cecropia* (*Cecropia* ants). Here, we show

that *Azteca andreae*, whose colonies build carton nests on
myrmecophytic *Cecropia*, is not a parasite of *Azteca–
Cecropia* mutualisms nor is it a temporary social parasite of
A. alfari; it is, however, a temporary social parasite of *A.
ovaticeps*. Contrarily to the two mutualistic *Azteca* species
that are only occasional predators feeding mostly on
hemipteran honeydew and food bodies provided by the
host trees, *A. andreae* workers, which also attend hemi-
pterans, do not exploit the food bodies. Rather, they employ
an effective hunting technique where the leaf margins are

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fringed with ambushing workers, waiting for insects to alight. As a result, the host trees' fitness is not affected as *A. andreae* colonies protect their foliage better than do mutualistic *Azteca* species resulting in greater fruit production. Yet, contrarily to mutualistic *Azteca*, when host tree development does not keep pace with colony growth, *A. andreae* workers forage on surrounding plants; the colonies can even move to a non-*Cecropia* tree.

Introduction

Ant–plants or "myrmecophytes" live in mutualisms with a limited number of plant-ants that they shelter in domatia (i.e., hollow branches or thorns and leaf pouches) and usually provide with food through extra-floral nectar and/or food bodies (FBs). In turn, plant-ants protect their host myrmecophytes from several kinds of enemies, particularly defoliators that they eliminate through their predatory and/or territorial behavior (Dejean et al. 2007; Rico-Gray and Oliveira 2007). Also, many plant-ants attend sap-sucking hemipterans for their honeydew.

Because the transmission of this mutualism is horizontal, the partners need to renew their association at the sapling stage of the plant and each time a guest colony dies as in most cases myrmecophytes live longer than their mutualist ants (except for those plant-ants that have evolved a strategy of secondary polygyny to ensure longer colony life spans). This situation permits other ant species to short-circuit these associations and to exploit the rewards provided by the plant whilst providing nothing in return (Rico-Gray and Oliveira 2007). These species are called "cheaters" (i.e., having evolved from former mutualists) or "parasites" of the mutualism (i.e., exploiters with no mutualistic ancestor) (Janzen 1975; Gaume and McKey 1999; Bronstein 2001; Raine et al. 2004; Clement et al. 2008; Heil et al. 2009; Kautz et al. 2009; see also Wilkinson and Sherratt 2001).

We studied the ecology and behavior of *Azteca andreae*, which is specifically associated with two myrmecophytes: *Cecropia obtusa* and *Cecropia palmata* (Cecropiaceae). In the area studied in French Guiana, these *Cecropia* house colonies of two plant–ant species, *Azteca alfari* and *Azteca ovaticeps*, in their hollow trunks and branches, and provide them with glycogen-rich FBs and lipid-rich pearl bodies (see Davidson 2005; plant-ants associated with *Cecropia* are frequently called "*Cecropia* ants"). Like other *Azteca*, associated or not with myrmecophytes, the workers of these two mutualistic species prey on insects landing on the foliage of their host trees (Cabrera and Jaffe 1994; Dejean et al. 2009). *A. andreae* workers, however, build external, ovoid carton nests, and, rather than exploiting the FBs furnished by their host *Cecropia*, they frequently hunt large prey by ambushing side-by-side beneath the leaf margins, mandibles wide open (Fig. 1; Dejean et al. 2010). Also, *A. andreae* belongs to the *aurita* group that is composed of species thought to be temporary social parasites of other

Fig. 1 a A recently captured wasp is spread-eagled by a group of workers as nestmates begin to replace them in ambushing along the leaf margin. **b** Photo showing the beginning of the construction of a new *A. andreae* nest just under the crown of leaves while the old one is still being used; indeed, the nest position changes as the trees grow. Note that most of the leaf margins are fringed with ambushing workers. **c** Illustration of the technique used to evaluate the number of *A. andreae* workers per centimeter of leaf margin: we photographed the workers ambushing from beneath the *Cecropia obtusa* leaves while cautiously placing a ruler 1–2 cm away from the leaf margins so as not to perturb the workers
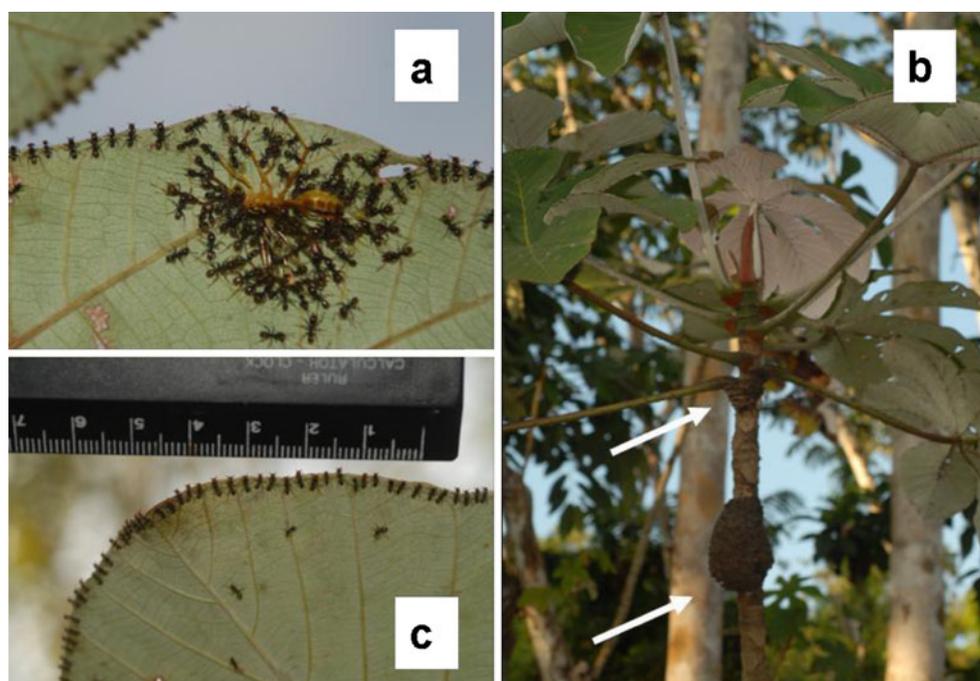
Azteca species due to the small size of the queens (Longino 2007; Guerrero et al. 2010), although this still remains to be demonstrated. In both temporary social parasitism and inquilism (or permanent parasitism), the queens are relatively small. In temporary social parasitism, the newly mated queens must find and sneak into a colony of the host species, and be adopted. Then, the original host queen is killed by the intruder or by her own workers. As the parasitic queen lays eggs and its brood develops into workers, there is an intermediary step consisting of a mixed parasite–host colony. Later, as the host workers are not replaced, the colony comes to consist entirely of the parasitic queen and her offspring (Hölldobler and Wilson 1990).

We sought to determine in this study if *A. andreae* is a true temporary social parasite of one or both *Cecropia* ants, if it is a parasite of the mutualism between these *Azteca* species and the myrmecophytic *Cecropia*, or both a temporary social parasite and a parasite of the mutualism. We therefore examined (1) the comparative number of *C. obtusa* and *C. palmata* sheltering *A. andreae* carton nests in the area studied, and if colonies can be sheltered by trees other than myrmecophytic *Cecropia*; (2) the size of the *A. andreae* colonies based on the size of their host tree, if the worker caste is polymorphic and if all kinds of workers are involved in hunting and thus play a role in protecting the plant foliage; (3) if young *A. andreae* colonies can form mixed colonies with *A. alfari* and/or *A. ovaticeps* (showing that *A. andreae* is a social parasite); (4) if *A. andreae* colonies protect their host *Cecropia* foliage from defoliators; and (5) if these colonies affect their host plant's fitness as evaluated through fruit production.

Materials and methods

Study site and model system

This study was conducted between 2004 and 2009 in secondary forest formations in French Guiana near the Petit Saut dam (5° 03′ 39″ N – 53° 02′ 36″ W), along Route N°1 between Kourou (5° 09′ 35″ N – 52°39′01″W) and Sinnamary (5° 22′ 60″ N – 52° 57′ 0″ W), along the road to Kaw Mountain (between 4° 43′ 60″ N – 52° 17′ 60″ W and 4° 38′ 20″ N – 52° 06′ 30″ W), along the last kilometer of the dirt road leading to the *Auberge des Chutes Voltaire* (5° 29′ 27″ N – 54° 02′ 16″ W), and along 1 km of Route N°1 west of Iracoubo (5° 28′ 60″ N – 53° 13′ 0″ W). We recorded a total of 145 *A. andreae* nests for which the host tree was identified and measured. The location of each *A. andreae* colony was noted.

We first verified the number of *A. andreae*, *A. alfari*, and *A. ovaticeps* nests on 3,544 *C. obtusa* (widely distributed)

and 1,432 *C. palmata* (restricted to the white sands found along coastal areas) more than 4.5 m in height growing alongside the roads. We then surveyed 105 *C. obtusa* near Kaw Mountain (where *C. palmata* is absent), and 129 *C. palmata* from an area situated west of the village of Iracoubo (white sands; *C. obtusa* is very rare). We incited the ants to leave the domatia by tapping the tree trunk with the flat side of the blade of a machete; we then used an aspirator to gather some of the workers for further identification. When, exceptionally, no workers left the domatia, we cut open the trees with the machete to gather the ants. Voucher specimens were deposited in the *Laboratório de Mirmecologia*, CEPEC-CEPLAC, Itabuna, Bahia, Brazil.

Because *C. obtusa* is dioecious, we verified if *A. andreae* colonies shelter in both male and female trees during the period when the trees bear inflorescences (45 trees examined).

A. andreae colonies on trees other than myrmecophytic cecropia

As suggested by Longino (2007) for *Azteca schimperi*, another species in the *aurita* group, we hypothesized that *A. andreae* colonies can leave their host *Cecropia* tree to build a new nest on a non-*Cecropia* tree in the surroundings. We tried to trigger this phenomenon by cutting some leaves off of eight *C. obtusa* sheltering an *A. andreae* nest, and then verified if the colonies later moved to a nearby tree. Reciprocally, we connected the trunk of ten non-*Cecropia* trees sheltering an *A. andreae* nest (that had moved naturally or during the previous experiment) to that of a *Cecropia* situated in the area (1.5–6 m further away) using a branch whose extremities were attached to both trunks. Then, we cut several branches off of the host tree and verified after 4 weeks if the colony had moved to the *Cecropia*.

Size and composition of the *A. andreae* colonies

To estimate the population sizes of the *A. andreae* colonies, first we gathered nests from 25 *C. obtusa* trees. The carton nests plus the hollow tree branches were placed inside large plastic bags to ensure the capture of the maximum number of workers. The plastic bags were then transported to the laboratory, and placed in a refrigerator at 4°C for ca. 3 h. To evaluate colony composition, the branches, and then the nests, were taken out of the refrigerator, completely opened, and we used smooth forceps to gather the numbed workers, winged sexuals, and queens and put them into a plastic vial containing 75° ethanol. The individuals were counted all throughout the process. Also, 21 additional small *A. andreae* nests (smaller than 10×6.5 cm; height × diameter)

and their host *Cecropia* trees were opened to look for the presence of mixed colonies.

In order to verify if polymorphism in the worker caste plays a role in the distribution of their tasks, we selected three *C. obtusa* sheltering a medium-sized *A. andreae* carton nest (colonies 12, 14, and 16 from Table 1) from which we cut two leaves whose margins were fringed with ambushing workers, and put them into a large plastic bag. We then gathered the nests and put each of them into a plastic bag. Transported to the laboratory, the plastic bags were placed in a refrigerator for ca. 3 h permitting us to randomly sample 500 workers from each nest, and 100 of the corresponding hunting workers from the leaves (or a total of 1500 and 300 workers, respectively). We weighed each worker with a microscale (Mettler® AE 260) and compared the mean weight (±SE) of the workers from the two lots using the unpaired *t* test.

Hunting plays a major role in the biology of *A. andreae*, as demonstrated by the fact that the margins of all of the leaves of a host tree are very frequently fringed with ambushing workers (Dejean et al. 2010). But just how many workers per colony are involved in this ambushing effort? In an attempt to answer that question, we used the three colonies mentioned above to compare the total number of workers per colony with the theoretical number of workers likely to hunt side-by-side beneath the margins of all of the leaves on each tree. To evaluate this theoretical number, we first calculated the density of the ambushing workers by placing a ruler ca. 1.5 cm from the leaf margins and photographed the ants (Fig. 1c) resulting in ca. 4.4 workers per cm (*N*=80; Dejean et al. 2010). We then cut off all of the leaves from each corresponding tree to measure the length of their margins using a measuring tape. The total length of the leaf margins and the density of the

Table 1 Composition of the colonies according to the size of their nests and of their host trees

	Size of the colonies and their host trees							
	No. workers	No. males	No. winged females	Physogastric queen presence	Size of the nests (h × lcm)	Height of trees (m)	No. leaves	No. *Azteca ovaticeps* workers
1	30,899	6,888	1,468	Yes	31×15	19	55	–
2	22,600	Pupae	Pupae	Yes	16.5×13.5	7	22	–
3	22,240	255	840	Yes	17×12	18	35	–
4	21,200	0	0	Yes	15×13	6	7	–
5	19,019		0	Yes	17×10.5	15	33	–
6	18,900	0	0	Yes	10×9+5×3	7	7	–
7	18,250	521	1	Yes	17.5×12.5	6	6	–
8	15,230	79	16	Yes	16.5×11.5	11	28	–
9	14,990	0	0	Yes	14.5×12	10	8	–
10	11,500	46	0	Yes	15×11.5	7	27	–
11	10,550	0	0	Yes	11.5×6.5	6	8	–
12	9,800	64	0	Yes	10×5	6	10	–
13	8,360	61	0	?	18×10	13	23	–
14	6,015	0	0	Yes	14×10.5	7	29	–
15	5,950	0	1 pupae	Yes	6.5×5	6	14	133
16	5,750	0	0	Yes	11×8	7	18	–
17	5,660	0	0	Yes	9×5.5	6	7	510
18	5,620	0	0	Yes	7.5×4.5	4.5	29	–
19	5,370	2	1	Yes	9×6.5	7	32	690
20	4,740	0	0	Yes	8×5	5	11	312
21	4,314	1	1	Yes	9×6.5	6	9	–
22	4,200	0	0	Yes	8×6.5	15	37	–
23	4,101	1	0	Yes	7×5.5	12	5	–
24	2,280	0	0	Yes	7.4×4.6	5	9	–
25	2,192	1	0	Yes	8×6	6	13	64

Each nest plus hollow internodes were gathered from the host trees, put into plastic bags, transported to the laboratory, and put into a refrigerator for ca. 3 h. Then, the nests and internodes were completely opened in the laboratory, and the individuals counted by the co-authors

ambushing workers permitted us to obtain their theoretical number.

Impact of *A. andreae* on their host tree foliage and fitness

We compared the defoliation of *C. obtusa* trees of similar sizes and sheltering different ant species during two surveys conducted 9 months apart. The results were very similar, so that we present only those from the second survey. Indeed, these results give us an idea of the history of the defoliation over the preceding ca. 18 months corresponding to the lifespan of the *C. obtusa* leaves (noted by tagging young leaves; AD, personal observation). The surveys were conducted in May 2007 between Kourou and Sinnamary during "normal" conditions involving several defoliating insects prone to attacking the leaves, but generally expulsed by the workers when discovered, and in June 2008 along the road to Kaw Mountain during a proliferation of *Dircema nigripenne* (Chrysomelidae: Galerucinae). Like for some other galerucine species (Jolivet 1996), the larvae can live and feed on *Azteca*-inhabited *Cecropia* trees.

We defined four levels of defoliation (when present, the youngest, still red–brown leaves were not taken into consideration): (1) not attacked: leaves intact or only defoliated to less than 5% of their surface; (2) slightly attacked: several leaves were attacked, and 10% to 50% of their surface was destroyed; (3) somewhat attacked: ca. all of the leaves were attacked, and 10% to 50% of their surface was destroyed; and (4) very attacked: all of the leaves were attacked, and more than 50% of their surface was destroyed. The results were compared using the Kruskal–Wallis test followed by a Dunn's *post hoc* test for multiple comparisons.

To evaluate the impact of the compared ant species on the fitness of the trees, we used direct observation to study fruit production by the 3,544 *C. obtusa* more than 4.5 m tall growing alongside the roads.

For statistical comparisons (Chi-square test, unpaired *t* test, Kruskal–Wallis and Dunn's tests), we used GraphPad Prism 4.03 software.

Results

Tree species hosting *A. andreae* colonies

Only a small percentage of the *Cecropia* spp. trees sheltered *A. andreae*. During the first series of observations, we noted 77 out of 3,544 (2.17%) and eight out of 1,432 (0.56%) *A. andreae* nests on *C. obtusa* and *C. palmata*, respectively; the difference is significant ($\chi_{Yates}^2=14.88$; $P<0.0001$). The in-depth survey conducted in Iracoubo and at Kaw Mountain also showed that significantly more *A. andreae* nests are sheltered by *C. obtusa* than by *C. palmata* ($\chi_{Yates}^2=46.53$; 6 *df*; $P<0.0001$), while we noted six ant species likely to shelter in the hollow branches of *C. palmata* and *C. obtusa* (Fig. 2). When the trees bore inflorescences, we noted that *A. andreae* nested similarly on male and female *Cecropia* (23 installed on males, 22 on females; $N=45$).

Out of the 145 *A. andreae* nests recorded in total during this 6-year-long study, all but nine (93.8%) were found on a *C. obtusa* or a *C. palmata* whose height varied from 4.5 m to ca. 25 m. We noted 35.9% of the *A. andreae* nests on *Cecropia* 4.5–8 m tall, 27.6% on trees 8–12 m tall, 17.9% on trees 12–16 m tall, and 12.4% on trees 16–25 m tall. The latter case corresponds to the tallest ca. 30-year-old trees that sheltered the largest carton nests containing numerous winged sexuals (see tree No. 1 in Table 1). Although the nests are rebuilt as the trees grow (Fig. 1b), this shows that the association can likely persist for a long time. The nine non-*Cecropia* trees bearing an *A. andreae* colony belonged to different species frequently found in pioneer formations (i.e., *Chrysophyllum argen-*

Fig. 2 The different ant species sheltered by *Cecropia palmata* (*N*=105) and *Cecropia obtusa* (*N*=129). *Azteca sericeasur* is a polydomous nesting species whose queens nest in live stems, near the base, while workers and brood are found in carton pavilions where they also attend hemipterans (Longino 2007). *Camponotus balsami* and *Daceton armigerum* also nest in hollow branches; the former, nocturnal, is frequent in pioneer formations; while the latter, diurnal, is rather a canopy species
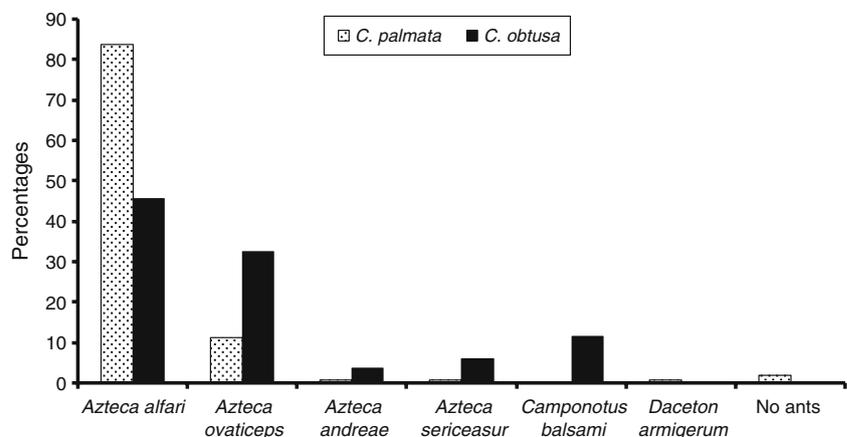
teum, Sapotaceae; *Miconia* sp. and *Bellutia* sp., Melasto-mataceae; *Vismia guianensis*, and *V. latifolia* Clusiaceae).

Azteca andreae colonies moving to trees other than myrmecophytic *Cecropia*

We witnessed five cases where there was the progressive occupation of the foliage of the trees surrounding a *C. obtusa* bearing an *A. andreae* colony, and then the beginning of the construction of a new nest on one of these trees. In all cases, the host Cecropia had lost branches due to an accidental event. The workers continued to hunt on the foliage of the abandoned *Cecropia* tree as well as on the new host tree and the surrounding vegetation; the same was true for their exploitation of hemipterans.

We successfully reproduced this phenomenon by cutting several leaves off of eight *C. obtusa*. The same result was obtained by cutting several leaves off of 10 non-*Cecropia* trees sheltering an *A. andreae* nest (four had moved naturally, the six others belong to the previous experiment) whose trunks were connected with those of a neighboring *C. obtusa* using a long branch. In both experiments, the new nests were built in ca. 3 weeks.

Size and composition of *A. andreae* colonies, and the search for the host *Azteca* species

The largest *A. andreae* nest contained a physogastric queen (i.e., whose gaster was swollen because the ovaries were enlarged), more than 30,000 workers, numerous eggs, larvae and pupae, and winged sexuals (Table 1). The presence of a physogastric queen (see photo in Guerrero et al. 2010), was recorded in 24 out of the 25 nests, plus five other nests opened during preliminary studies.

Five among the smallest *A. andreae* nests also contained *A. ovaticeps* workers but no queen from this species (Table 1), pointing to the existence of mixed colonies. We therefore opened 21 additional small *A. andreae* nests, and noted the occurrence of *A. ovaticeps* workers in 16 of them, resulting in a total of 21 mixed *A. ovaticeps–A. andreae* colonies recorded. In no case was the co-occurrence of *A. alfari* and *A. andreae* workers detected.

To test the hypothesis that *A. andreae* is an exclusive parasite of *A. ovaticeps*, we estimated the probability, p, that *A. andreae* parasitizes *A. alfari*, based on our data. We first assumed that *A. andreae* parasitizes *A. alfari* or *A. ovaticeps* according to a Bernoulli process with parameter p. This assumption implied that *A. alfari* and *A. ovaticeps* colonies were randomly distributed among *C. obtusa* trees and with similar abundances. Hence, K, the number of *A. alfari* colonies parasitized among the N colonies parasitized by *A. andreae* followed a binomial distribution with parameters N and p. Taking into account the data ($x = 0$

and $n = 21$), the likelihood of the parameter, p, was then given by $L(p; x = 0, n = 21) = (1-p)^{21}$. According to Bayes' formula, the density, f, of the probability distribution of p conditionally to the data is proportional to $L(p; x = 0, n = 21)$. As the integrate of a density function equals one, we had to normalize L by its integral over $[0;1]$ (equal to $1/22$) to get $f(p|x = 0; n = 21) = ((1-p)^{21}) * 22$. From f we computed the usual estimators of p: mean$=0.0435$, median$=0.0310$ and the 95th confidence interval $[0; 0.1273]$. Because the abundance of *A. alfari* and *A. ovaticeps* colonies was assumed to be similar in our model even though *A. alfari* is more frequent than *A. ovaticeps* (Fig. 2), the estimators for p were conservative. It is therefore likely that *A. andreae* only parasitizes *A. ovaticeps*.

Note that during earlier studies examining the territoriality of *A. alfari* and *A. ovaticeps*, we found four mixed *A. andreae–A. ovaticeps* colonies sheltering in the hollow trunks of young *C. obtusa* trees that we interpreted to be the step before the construction of an external, carton nest by *A. andreae* workers. While *A. ovaticeps* workers gathered FBs, *A. andreae* workers ignored them.

Hunting workers and polymorphism in the worker caste

Through their mean weight, we also noted that hunting workers are significantly heavier (and larger) than those gathered from inside the nests and belonging to the internal service: 1.393 ± 0.0197 mg ($N = 300$) versus 0.68 ± 0.011 ($N = 1500$); unpaired t test: $t = 26.8$; 1798 df; $P < 0.0001$.

After observing three *Cecropia* trees sheltering an *A. andreae* colony during several days and noting which leaves had their entire margin occupied by ambushing workers, we cut off these leaves, measured the total length of their margins and evaluated the number of workers likely to hunt on them based on 4.4 workers per cm. Compared to the total number of workers in the colonies, we noted that up to 85.2% of the workers can be involved in ambushing side-by-side (colony 10 from Table 1: 8350 ambushing workers versus 9800 workers in total, 85.2%; colony 12: 4800 versus 6015, 79.8%; colony 14: 4300 versus 5750, 74.8%).

Also, the sum of the margins of all of the leaves on the tree (or the total length of the leaf margins) will determine the maximum number of workers that can hunt at one time. In most of the cases, the total number of workers in the colony was lower than the theoretical number of workers that can ambush side-by-side along the total length of the leaf margins of the corresponding trees. Thus, the hunting workers ambushed along the margins of only a part of their host tree leaves (Fig. 3). This is particularly true for *A. andreae* colonies having likely colonized large trees (horizontal series of diamonds; Fig. 3). On the contrary, when the total number of workers
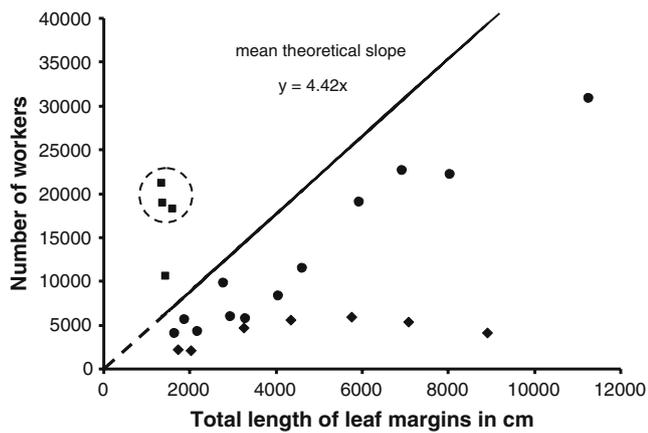
Fig. 3 Ratio between the number of workers in 23 nests opened and the total length of the leaf margins available in each case. The mean theoretical slope corresponds to the number of workers that can hunt side-by-side along the total length of the leaf margins based on 4.4 ants per cm. Three groups of colonies can be distinguished. For the four colonies situated above the slope (*squares*), particularly the three-circled, part of the workers foraged on the surrounding foliage. For the colonies below the slope, the workers only occupy a fraction of the total length of the leaf margins when ambushing. The number of workers is correlated to the total length of the leaf margins for the colonies represented by a *circle*. They likely correspond to colonies whose size increased with that of the host trees. Colonies represented by *diamonds* correspond to small colonies nesting on comparatively large trees; they probably parasitized large *Azteca ovaticeps* colonies

Fig. 4 Scatter plot illustrating the relationships between the number of workers per colony and the size of the host tree (*diamonds*). The four colonies represented by circles correspond to nests installed on relatively small trees, while the three colonies represented by *triangles* correspond to small nests installed on relatively tall trees
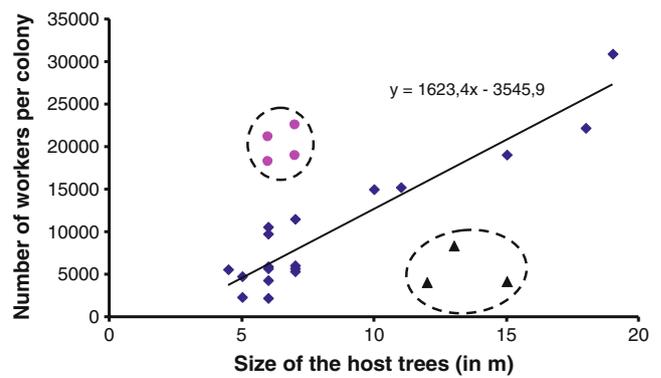
in the colony was by far superior to this theoretical number, a part of the hunting workers ambushed for prey on the foliage of the surrounding plants, something noted for the three colonies circled in Fig. 3 (corresponding to colonies Nos. 4, 6 and 7 in Table 1).

We experimentally reproduced this situation by cutting off three leaves from each of eight *Cecropia* trees bearing an *A. andreae* nest. After 4 days, some of the hunting workers ambushed on the foliage of the surrounding vegetation (that they had reached from the ground), illustrating a relationship between the size of the colony and the total length of the leaf margins. Later, they also attended hemipterans on the surrounding trees.

Relationship between the size of *A. andreae* colonies and the size of the host trees

The smallest tree bearing an external *A. andreae* carton nest was 4.5 m tall, indicating that colonization probably began while the host *A. ovaticeps* colony was relatively populous. Later, as the trees grow, the workers build new nests just under the new crowns using materials from the old nest whose size decreases as a result (see nest N° 5, Table 1; Fig. 1c).

The scatter plot displaying the relationship between the number of workers per colony and the size of the host tree (Fig. 4) shows a positive relationship for 18 out of the 25 *A. andreae* colonies. A cluster of outliers is represented by

four large colonies installed on relatively small trees (corresponding to Nos. 2, 4, 6, 7 in Table 1), but tree No. 2, which has several branches and numerous leaves, rather belongs to the previous case. The second cluster of outliers is represented by three small colonies sheltering on relatively tall trees (corresponding to Nos. 13, 22, 23 in Table 1). Except for tree No. 23 with only five leaves (in certain cases, the trees have a fast vertical growth), this might indicate that *A. andreae* can colonize even relatively large *A. ovaticeps* colonies sheltering on large trees.

Impact of *A. andreae* on their host tree foliage and fitness

Unexpectedly, *C. obtusa* individuals sheltering *A. andreae* colonies were significantly less defoliated than all of the others, whatever ant species they sheltered (Fig. 5). This was true both during 'normal conditions' and during a proliferation of the galerucine chrysomelid *D. nigripenne*, which is a *Cecropia* defoliator whose larvae are well adapted to the presence of *A. alfari* and *A. ovaticeps*.

While verifying the impact of *A. andreae* on the fitness of *C. obtusa*, we noted significantly more fruits on trees more than 4.5 m tall sheltering *A. andreae* colonies than on those sheltering an *A. alfari* or an *A. ovaticeps* colony (the two latter species pooled; the tree size distribution is similar): 52.25% ($N=77$) versus 28.32% ($N=3467$); $X_{Yates}^2=11.61$; $P<0.001$.

Discussion

Parasitism of the *Azteca-Cecropia* mutualisms versus the social parasitism of *Cecropia* ants

In this study we show that *A. andreae*, whose colonies build carton nests on myrmecophytic *Cecropia*, is not a parasite of *Azteca-Cecropia* mutualisms, but a temporary
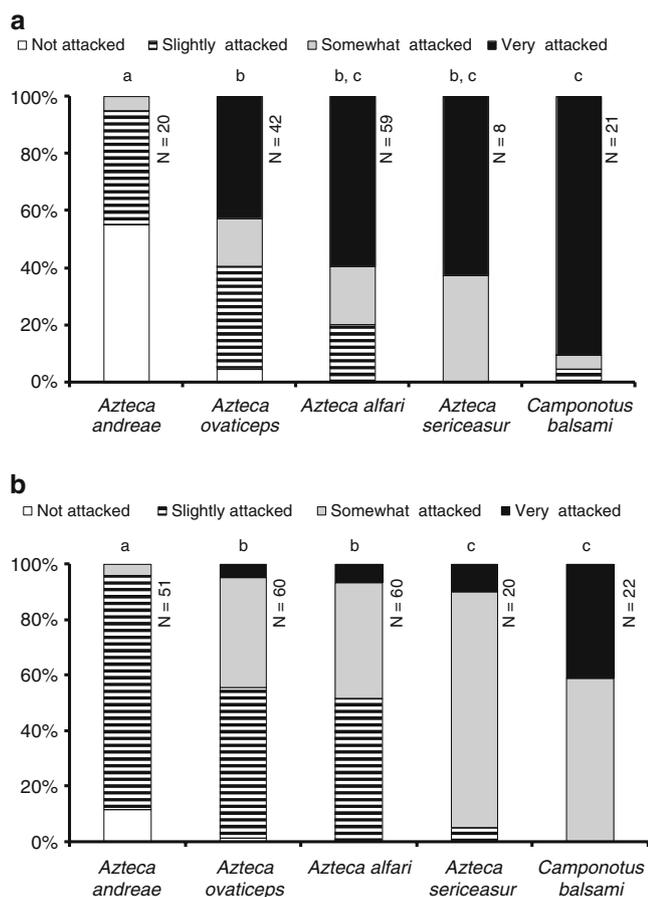
Fig. 5 Percentage of defoliation of 8–10-m-tall *Cecropia obtusa* according to the ant species sheltered. **a** Percentage of defoliation during "normal" conditions involving several defoliating insects such as adult locusts including large individuals, and several chrysomelid species that are generally attacked or intimidated by *Azteca* workers. **b** Percentage of defoliation during a proliferation of *Dircema nigripenne*, a galerucine chrysomelid. Statistical comparisons. Kruskal–Wallis test: $H_{150}^4=88.49$ and $H_{213}^4=57.25$ for **a** and **b**, respectively; $P<0.0001$ in both cases. Dunn's *post hoc* test: different letters indicate significant differences at $P<0.01$

social parasite of *A. ovaticeps*, one of the two mutualistic *Azteca* species of myrmecophytic *Cecropia* growing in the area studied. Indeed, parasites of plant–ant/myrmecophyte mutualisms colonize the myrmecophytes, but without providing them with a 'reciprocal' service (generally in the form of protection from defoliating insects). Because of major defoliation, these myrmecophytes are also affected in their fitness as they produce fewer seeds than individuals sheltering the appropriate plant-ants (Fischer et al. 2002; Heil and McKey 2003; Heil et al. 2004; Rico-Gray and Oliveira 2007).

Here we show that, contrarily to parasites of the mutualisms, *A. andreae* colonies protect their host *Cecropia* trees more efficiently than do *A. alfari* or *A. ovaticeps*. This was particularly true during the proliferation of a special-

ized chrysomelid beetle that is tolerated by *A. alfari* and *A. ovaticeps* in the area studied (see also Jolivet 1996), whereas it is eliminated by *A. andreae* workers. This protective action appears to be a by-product of the very effective group ambushing behavior of *A. andreae* workers permitting them to capture large insects (Dejean et al. 2010) and to the frequent presence of numerous workers ambushing on their host tree foliage (this study). This compensates the absence of purely defensive behavior and of an induced defense in *A. andreae* as opposed to *A. alfari* and *A. ovaticeps* (Dejean et al. 2008, 2009). Also, *A. andreae* does not affect the host tree's fitness; on the contrary, *Cecropia* trees bearing *A. andreae* nests produced more fruits than those associated with *Cecropia* ants.

A. andreae as a social parasite of *Azteca ovaticeps*

Occurrences of social parasitism are poorly documented for inter-tropical and sub-tropical ant faunas (Hölldobler and Wilson 1990). Most of the rare cases of Neotropical social parasites reported correspond to inquiline ants as the small queens were found while opening the nests of their host *Acromyrmex* (Sumner et al. 2004; De Souza et al. 2007), *Ectatomma* (Feitosa et al. 2008), *Pheidole* (Wilson 1984, 2003), *Pseudomyrmex* (Ward 1996), and *Solenopsis* (Calcaterra et al. 1999) species. The existence of temporary social parasites has been shown in two *Pseudomyrmex* species: small queens and colonies mixed with a host species (Ward 1996). Concerning the *aurita* group of *Azteca*, only the small queens provide an indication that the group is likely composed of social parasites (Longino 2007; Guerrero et al. 2010), something now demonstrated in this study for *A. andreae*, and explaining why this species is relatively infrequent. Although *A. alfari* is very frequently found in the same areas, *A. andreae* seems to exclusively parasitize *A. ovaticeps* with which it forms intermediate, mixed colonies. We found no *A. ovaticeps* workers after the *A. andreae* colonies reached a size of ca. 6,000 workers, whereas *A. andreae* individuals were no longer noted inside the domatia. It is likely that they are unable to recognize as such the prostomata, or thinner areas only recognizable to the founding queens and workers of *Cecropia* ants (Davidson 2005).

In general, when the abiotic conditions support *Cecropia* tree growth, the *A. andreae* workers limit their foraging activities to its foliage and can reach a large colony size and produce sexuals (see nest No. 1 in Table 1). Yet, when host tree development is slowed down due to exceptional conditions such as soil exhaustion, excessive dryness or the accidental breaking off of branches, the workers may be led to attend hemipterans and hunt on surrounding plants. The colonies can even move, and the workers build a new nest on a tree that can be a plant other than *Cecropia*; some

of these characteristics have been noted for *A. schimperi* (Longino 2007). Therefore, *A. andreae* is not an entirely obligate *Cecropia* inhabitant as are the mutualist *Azteca* species, and when their host *Cecropia* tree dies accidentally they are able to move to another tree nearby and so to persist. Yet, these cases remain exceptional and most *A. andreae* colonies grow as their host *Cecropia* tree grows. Also, when moving to a tree other than *Cecropia*, it becomes more difficult for *A. andreae* to easily catch large prey as the *Cecropia* leaves are larger than those of other trees in the area permitting numerous workers to ambush at one time, and their underside is particularly downy. This velvet-like structure combines with the hook-shaped claws of the *A. andrea* workers to act like a natural Velcro® that reinforces the group ambush strategy, allowing the workers to capture insects of up to 13,350 times their mean weight (Dejean et al. 2010).

As a social parasite of *A. ovaticeps*, however, *A. andreae* still takes the place of a true mutualist, so that one can wonder if the reduced herbivory and resulting higher fruit production that appears to be a by-product of its hunting behavior, does not hide other benefits furnished by true mutualistic *Azteca* species. Indeed, after the intermediate, mixed colony phase with *A. ovaticeps*, the *A. andreae* colonies build a carton nest, and then no longer use the *Cecropia* domatia. As such, the host *Cecropia* trees lose a part of the benefits they gain from their true mutualistic *Azteca* species: the provision of nutrients as noted for *C. peltata* (another myrmecophytic *Cecropia*) for which 93% of the nitrogen is derived from debris deposited in the domatia by its guest *Azteca* ants (Sagers et al. 2000). Plant-ants can supply myrmecophytes with nutrients. Beattie (1989) called this phenomenon "myrmecotrophy". This trait has major importance when nutrient availability is low, explaining why myrmecotrophy is frequent in epiphytes (Beattie 1989; Rico-Gray and Oliveira 2007). Because the level of nutrient availability for geophytes growing on poor tropical soils is also low and exacerbated further when sequestered by competing plants, myrmecotrophy has also been noted for myrmecophytic geophytes. This is the case for Malaysian rattan palms (Rickson and Rickson 1986), neotropical Melastomataceae (Cabrera and Jaffe 1994; Solano and Dejean 2004), *Piper* (Fischer et al. 2003), and *Cecropia* (Sagers et al. 2000). Therefore, the advantages that ants provide to their host myrmecophytes are notably greater when myrmecotrophy is added to biotic defence.

Nest-site selection

Nest-site selection by founding *A. andreae* queens implies that they find a *Cecropia* tree (there are two suitable species in the area studied) inhabited by the appropriate *Azteca* species. In the cases where this has been studied, the specificity of plant-ants for one myrmecophyte species is due to the genetically–determined attraction of founding queens toward this species, while in 'generalist' arboreal ants a familiarization process (contact with other plants during development) can override this genetically–determined attraction (Djiéto-Lordon and Dejean 1999a, b).

It is likely that the selection of adequate *Cecropia* trees by founding *A. andreae* queens is genetically determined; otherwise, the queens produced by colonies having moved to another tree species would be unable to select *Cecropia* due to the absence of an adequate familiarization process. The same is true for the selection of *A. ovaticeps* colonies (rather than *A. alfari*), as winged sexuals develop a long time after the mixed colony phase and so are never in contact with *A. ovaticeps* individuals (or only exceptionally; see colony 13, Table 1). The higher number of *A. andreae* colonies sheltered by *C. obtusa* can be explained by the fact that more *A. ovaticeps* are associated with this tree species than with *C. palmata* (Fig. 2).

In conclusion, we show that *A. andreae* is a temporary social parasite of the plant–ant *A. ovaticeps*, but is not a parasite of the *Azteca–Cecropia* mutualism. Indeed, (1) the workers do not take advantage of the rewards furnished by the host *Cecropia*, (2) contrarily to *Cecropia* ants, they can forage on other trees and their colonies can move, and (3) they even provide their host *Cecropia* with better protection from defoliators than do *Cecropia* ants, while enhancing their fitness. Two aspects of the biology of *A. andreae* are especially notable: its predatory strategy involving up to 85% of the colony's population of workers; and, like for plant-ants, nest-site selection is likely genetically determined with the life cycle obligatorily passing through an association with myrmecophytic *Cecropia* (and an adequate host-ant colony).

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