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Ant protection of a Heteropteran trophobiont against a parasitoid wasp

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Abstract Trophobiotic associations between *Catantopini* *rugosa* (Heteroptera; Plataspidae) and two ant species, *Camponotus brutus* (Formicidae) and *Myrmica opaciventris* (Myrmicinae), were recorded at the base of the trunks of *Bridelia micrantha* (Euphorbiaceae). The bug colonies were generally sheltered in pavilions built by the ants, but during periods of proliferation, part of the colonies developed outside the pavilions. We examined the protective activity of the two associated ant species against an encyrtid parasitoid wasp that parasitized egg masses of the bug. Egg masses situated outside the pavilions were significantly less parasitized on trees occupied by either ant species than on experimental trees where ants were excluded by a sticky barrier. Egg masses were significantly less parasitized on trees occupied by *C. brutus* than on those occupied by *M. opaciventris*, both inside and outside the pavilions. Moreover, they were less parasitized inside than outside the pavilions when protected by *C. brutus*. Inside the pavilions, parental care of the egg masses and first-instar nymphs was abandoned to the ants. In contrast, outside the pavilions, the females of *C. rugosa* guarded egg masses and clusters of first-instar nymphs. Furthermore, adults and last-instar nymphs grouped around and above first-instar nymphs, forming a protective shield. These groups formed and disintegrated daily, according to the attending ant's rhythm of activity.

Keywords Trophobiosis · Plataspidae · Formicidae · Encyrtidae

Introduction

Trophobiosis is a symbiotic relationship between ants and the insects, trophobionts, that they attend. The ants obtain honeydew from the trophobionts and in turn protect the trophobionts from their natural enemies (Gaume et al. 1998; Moog et al. 1998; Dejean et al. 2000a). Most of the known cases concern Hemiptera of the former suborder Homoptera (now Sternorrhyncha, Cicadomorpha, and Fulgoromorpha), or the Lycaenidae among the Lepidoptera, but trophobiotic relationships between ants and certain Heteroptera do exist. In particular, Plataspidae bugs have been noted to be sheltered in ant pavilions in Malaysia and Africa (Maschwitz and Klinger 1974; Maschwitz et al. 1987; Dejean et al. 2000b).

Until now, observations on ant-heteropteran associations concerned only ant attendance of the bugs. Information on the protective role of the ants has so far been lacking, except that ant protection was inferred when ants attended the bugs in pavilions. We therefore decided to experimentally test the protective role of two ant species against a parasitoid wasp (Chalcidoidea, Encyrtidae: *Ooencyrtus* sp.). Then, we studied the behavioral plasticity of adult bugs toward their eggs and first-instar nymphs. Finally, we compared this association with ant-attended Membracidae and Tettigometridae, two families belonging to the suborders Cicadomorpha and Fulgoromorpha, respectively. In the latter cases, females that clump their egg masses promote offspring aggregation. The survival of the nymphs depends on the number of individuals in the aggregations since larger groups of nymphs appear to be located sooner by ants and are consistently attended (Wood 1982; see also Dejean et al. 2000a). Additionally, there is often a shift in the protection of membracid and tettigometrid progeny from the female to the attending ants. Parental care can be restricted to egg guarding and the protection of first-instar nymphs, while associated ants guard later instars. Egg protection by ants can even surpass the females' activity, while nymph guarding is completely undertaken by the ants (Wood 1977; Bristow 1983; Dejean et al. 2000a).

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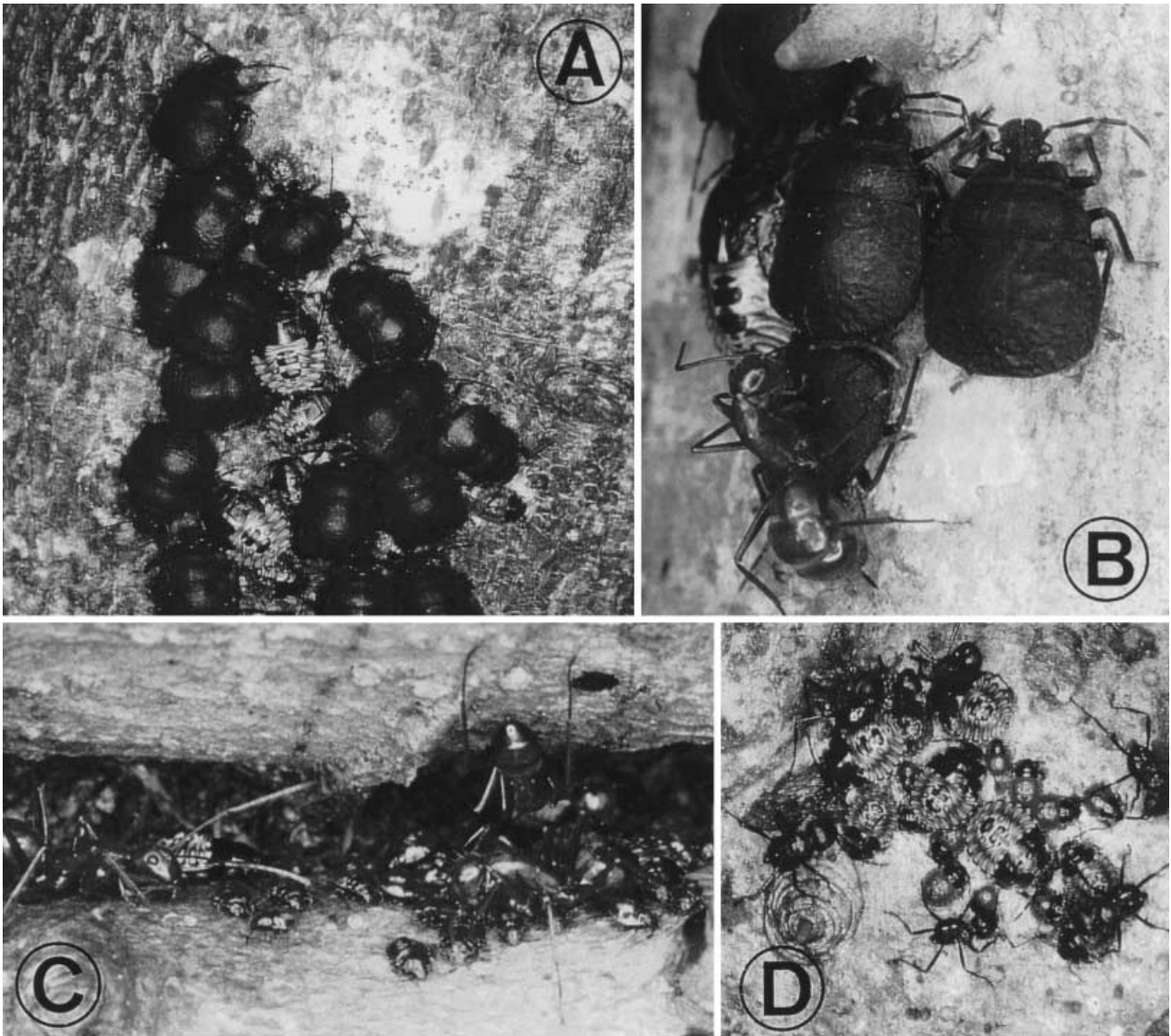


Fig. 1 **A** Adults forming a shield around and above nymphs. **B** Clusters of nymphs and adults disperse into small groups at night when attended by *Camponotus brutus* workers. **C** Inside the pavilions of *C. brutus*, nymphs are grouped by size. **D** *Myrmecaria opaciventris* workers (gaster under their thorax) attending nymphs of different stages outside the pavilions

Materials and methods

This study was undertaken in southern Cameroon from December 1987 to July 1993. *Caternaultiella rugosa* (Heteroptera; Plataspidae) was only noted on two euphorbiaceous trees (*Bridelia micrantha* and *B. grandis*) and was attended by two ant species, *Camponotus brutus* (Formicinae; nocturnal) and *Myrmecaria opaciventris* (Myrmicinae; diurnal) in carton pavilions built by the ants at the base of the tree trunks. During a proliferation of the bug population, part of the clusters of nymphs and adults developed outside the pavilions. In the latter case, *M. opaciventris* workers did not modify their rhythm of activity to attend these clusters, while *C. brutus* workers, normally nocturnal, attended them day and night (Mercier and Dejean 1996; Kenne and Dejean 1999; Dejean et al. 2000b). Along the Cameroonian rainforest edges, *Bridelia* trees

are relatively frequent pioneers that do not furnish shelter to ants in the form of domatia, nor do they produce extra-floral nectar. Nevertheless, they are protected by ants that tend coccids, stictococcids, or tettigometrids on their branches (Dejean, 2000).

To conduct experimental studies, we transported groups of both adults and nymphs of *C. rugosa* to the University campus and Mvoolier Valley, Yaoundé, two sites where both *B. micrantha* and ants associated with *C. rugosa* were previously noted. In both cases, the bugs were accepted and tended by the ants, and their populations proliferated. We were therefore able to easily observe the behavior of female bugs and attending ants toward the Heteropteran offspring.

The protection by ants of the bug egg masses against parasitoid wasps (Chalcidoidea, Encyrtidae: *Ooencyrtus* sp.) was studied during a period of proliferation of the bug on 30 *B. micrantha*. These trees were occupied by *C. brutus* or *M. opaciventris* (15 trees each) attending colonies of *C. rugosa* 40 cm or higher on their trunks (another part of the bug colonies was attended in pavilions in the root area). In each case, 10 trees with ants served as a control, while five other trees from which ants were excluded through the use of a sticky barrier (an 8-cm band encircling the base of the tree, brushed over with birdlime each week) formed the experimental lot. On the first day of experimentation, previously hatched egg masses laid outside the pavilions were scraped with a knife. One month later, we used a magnifying glass to

count the number of eggs, parasitized versus non-parasitized, in the new egg masses with hatched eggs. Parasitized eggs had a neat circular hole made by the emerging wasps when leaving the eggs, while others were more widely torn open by the hatching first instars. We also opened the pavilions of 20 *B. micrantha* (10 occupied by *C. brutus*, the other 10 by *M. opaciventris*), and we counted the number of eggs (parasitized vs. non-parasitized) in the egg masses situated inside and outside the pavilions.

Statistical analyses were made using a generalized linear model (GLIM 1986 with a Poisson error for the number of egg masses at three different heights on the trunks, and with a binomial error for the percentages of parasitized eggs according to the type of protection (no ants, *Camponotus*, *Myrmicaria*) and the location (inside/outside pavilion). Multiple comparisons among pairs of means were performed for the number of egg masses [(logarithm+1)-transformed data] and the percentages of parasitized eggs (arcsine-transformed data) (Bonferroni method; Statistix 1994).

Results

Bug protection of their offspring

When the population of the bug was low, only a few adults and last-instar nymphs were present outside the pavilions. The egg masses and most nymphs and adults, sheltered in the pavilions, were therefore under ant protection. The 38 unhatched egg masses recorded in 44 pavilions were never surmounted by a female bug. Nymphs were generally grouped by size (Fig. 1), while adults, depending on the cases, were dispersed or grouped in the pavilions.

During the proliferation of the bug, we noted that egg masses laid near the pavilions were rapidly covered by the ants with carton, but not those laid more than 30 cm from the initial pavilions (42 observations recorded). Moreover, numerous clusters of nymphs were attended by ants outside the pavilions. As a result, part of the population remained in the pavilions while the rest developed outside. Outside the pavilions, female bugs clumped the egg masses (19–67 eggs; 38.04 ± 1.75 eggs per mass; $n=70$) mainly at the base of the tree trunks (Fig. 2). Egg guarding was restricted to females that placed themselves above or just touching their offspring (28.3% and 71.7%, respectively; $n=272$). Brood-guarding females never left their clutch, even when experimentally disturbed by a grass stalk, and secreted a foul-smelling secretion from the metathoracic glands (absent in the nymphs) through small openings on either side of the thorax, a general characteristic in Heteroptera.

First- and later-instar nymphs were always guarded and probably protected by several last instars, adults, and associated ants. In all cases, in the absence of attending *C. brutus* workers on a cluster, several adults of both sexes guarded the nymphs diurnally, outside the pavilions. They formed a shield by placing themselves above and around the nymphs (Fig. 1). Last instars sometimes also behaved similarly vis-à-vis first instars. Nocturnally, on trees occupied by *C. brutus*, nymphs and adults from the same clusters dispersed into smaller groups when they were attended by several ants (Fig. 1). The nymphs were entirely protected by the workers. There is therefore daily modification of the distribution of the bug in-

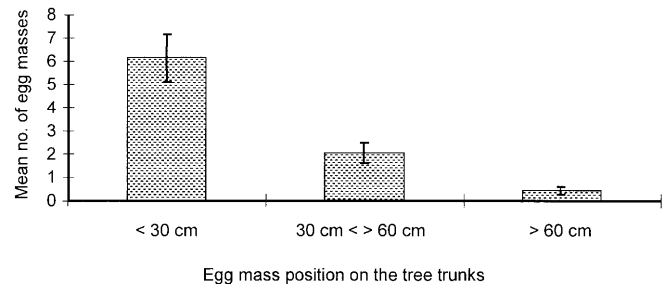


Fig. 2 Comparisons between the number of egg masses (mean±SE) in the first 30 cm, the next 30 cm, and the rest of the trunk of 20 *Bridelia micrantha* trees during a period of bug proliferation. The distribution of egg masses differed significantly along the trunk ($\chi^2=92.2$, $df=2$, $P<10^{-5}$). Egg masses were more frequent at the base of the trunk, near the ant nest, than higher up. All comparisons between the three positions resulted in significant differences ($P<0.01$)

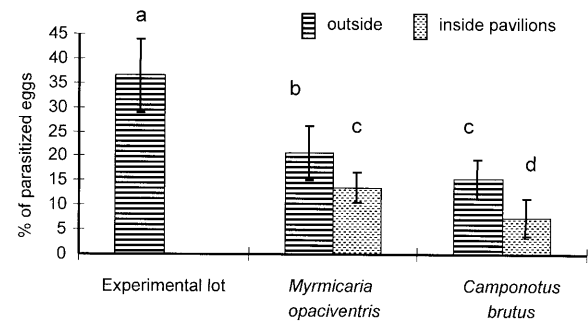


Fig. 3 Comparisons of the percentages of eggs parasitized (mean±SE) outside the pavilions when ants were excluded (experimental lot), when attended by *Myrmicaria opaciventris* or *Camponotus brutus*, and outside versus inside the pavilions. The percentages of parasitized eggs were significantly higher when ants were excluded than when present ($\chi^2=35.4$, $df=1$, $P<10^{-5}$), outside than inside ant pavilions ($\chi^2=11.6$, $df=1$, $P=0.0007$), and when the bugs were attended by *M. opaciventris* rather than *C. brutus* ($\chi^2=9.96$, $df=1$, $P=0.0016$). The comparisons between cases represented by different letters resulted in significant differences ($P<0.05$)

dividuals in these clusters according to the activity of *C. brutus* (see Dejean et al. 2000b).

Ants' effect on Encyrtidae parasitoid wasps

In the experimental lot, where ants were excluded, the proportion of parasitized eggs was significantly higher than in the control lots (presence of ants), indicating that ants provided protection against the encyrtid wasps (Fig. 3). In the presence of ants, regardless of species, the percentages of eggs parasitized by wasps were significantly higher outside than inside the pavilions (Fig. 3). The two ant species achieved different levels of protection: significantly more eggs were parasitized on trees occupied by *M. opaciventris* than on those occupied by *C. brutus*, both outside and inside the pavilions. *C. brutus* workers provided better protection of the bug eggs than did *M. opaciventris*, so that the lowest percentage of parasitized eggs was noted inside the *C. brutus* pavilions (Fig. 3).

Discussion

The relationships between *C. rugosa* and both *C. brutus* and *M. opaciventris* are truly mutualistic. The ants receive honeydew from the bug (Dejean et al. 2000b) while the bugs are protected by the ants against parasitoids in a manner similar to that for honeydew-producing insects of the former suborder Homoptera (Bradley 1973; McEvoy 1979; Bristow 1984; Buckley 1987, 1991; Sudd 1987; Jiggins et al. 1993). Nevertheless, the levels of mutualism differed according to the ant species involved: the degree of egg mass protection against parasitoid wasps was significantly higher in associations with *C. brutus* than with *M. opaciventris* both inside and outside the pavilions. Differences in the efficiency of protection of the same trophobiont by different attending ant species have already been shown (Buckley 1991; Novak 1994; Hübner and Völkl 1996).

The pavilions are not an absolute protection against this parasitoid wasp, despite the presence of associated ant species; nevertheless, the percentages of parasitized eggs were lower inside than outside the pavilions. Pavilions built by ants are known to provide protection to Sternorrhyncha and Cicadomorpha from weather and enemies, but certain coccinellids and parasitoids enter the pavilions thanks to morphological and behavioral adaptations or through chemical camouflage (Beattie 1985; Buckley 1987; Hölldobler and Wilson 1990; Völkl and Mackauer 1993; Völkl 1994, 1995). This situation is therefore more complex than that recorded when *C. brutus* workers protected the egg masses of tettigometrids against parasitoid wasps, as tettigometrids are never sheltered in pavilions (Dejean and Bourgoïn 1998; Dejean et al. 2000a).

Dumping eggs in masses may increase the risk of parasitism, necessitating the active protection of these egg masses (Tallamy 1985, 1986; but see also Kaitala 1999). Moreover, females can also clump the egg masses. In ant-attended membracid species that are never sheltered in pavilions, the clumping of egg masses, by promoting offspring aggregations, favors their defense through ant attendance. The survival of nymphs depends on the number of individuals in the aggregations, since larger groups of nymphs appear to be located sooner by ants and are consistently attended (Wood 1982). The clumping of egg masses has also been noted for tettigometrids and lycaenid larvae and seems a general feature in ant mutualisms (Axen and Pierce 1998; Dejean et al. 2000a).

In ant-membracid or -tettigometrid associations, parental care can be partially or completely transferred to the ants (Wood 1977; Bristow 1983; Dejean et al. 2000a). In the present study, *C. rugosa* females were characterized by a strong behavioral flexibility. When sheltered in pavilions, they abdicated parental care to attending ants. When living outside the pavilions, they clumped their egg masses close to the pavilions that are situated around the base of the tree trunks. As a result, a part of their egg masses was rapidly covered by new pavilions. Nevertheless, females showed a defense-like behavior of egg masses and newly hatched nymphs when

egg masses were laid relatively far from the ant pavilions and so were never covered by shelters. Their behavior was in this case very similar to that described for acanthosomatid heteropterans (Mappes et al. 1995). This condition favors group living, and probably permits females to protect offspring situated far from the pavilions.

Living in a group, although increasing the risk of being detected, is generally considered as a selective advantage against generalist predators as it dilutes predation pressure and increases vigilance and defense (McEvoy 1979). Nevertheless, many predators and parasitoids specifically exploit ant-attended resources with strategies adapted to the attending ant species (Jiggins et al. 1993; Völkl and Mackauer 1993; Novak 1994; Völkl 1994, 1995; Hübner and Völkl 1996). In the case of *C. rugosa*, defense depends particularly on the structure of the group, where adults, with their very hard cuticle and their defensive products, form a shield above first-instar nymphs and around large nymphs situated at the center of the clusters. Adults of both sexes plus the nymphs themselves were also characterized by a strong behavioral flexibility as they clustered to form a shield above first-instar nymphs only in the absence of attending ants and passed from one structure to the other daily, according to the ant's rhythm of activity, when attended by *C. brutus* (see also Dejean et al. 2000b).

In conclusion, *C. rugosa* individuals generally live in mutualistic associations with the ants that protect them. These associations are as well-developed as those known between ants and hemipterans of the former suborder Homoptera. Nevertheless, this species has not lost the ability to protect its offspring. In the absence of ant protection, during proliferation of the bug populations, the females place themselves above or in contact with their offspring, and groups of adults and last instars form a shield around or above younger individuals.

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