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Ants as selective agents on herbivore biology: effects on the behaviour of a non-myrmecophilous butterfly

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Summary
1. Larvae of the nymphalid butterfly *Eunica bechina* feed on young leaves of *Caryocar brasiliense*, a shrub of the Brazilian savannah that is frequently visited by nectar-gathering ants.
2. Eggs are not removed by ants, but ant occupation on plants affects the oviposition behaviour of the butterflies. Adult females avoid laying eggs on highly visited plant locations and visual cues were demonstrated to mediate oviposition by the butterflies. Plant branches with artificial rubber ants were significantly less infested than control branches with rubber circles. This is the first demonstration that ant presence per se can be enough to produce an avoidance response by ovipositing females in a non-myrmecophilous butterfly.
3. Larval mortality was strongly affected by the level of ant visitation to the host plants, and vulnerability to ant predation decreased with larval size.
4. Stick-like frass chains constructed by the larvae at leaf margins were demonstrated to be a safe refuge against ant attacks on the host plant. Live termites placed on leaves were attacked by foraging ants in significantly greater numbers than those placed on the frass chains.
5. It is concluded that the behavioural biology of both immature and adult *Eunica bechina* is finely linked with the utilization of a host plant where ant visitation patterns strongly affect larval survival.

Key-words: ant–butterfly interaction, ant predation, extrafloral nectaries, oviposition behaviour, plant–herbivore relationships.


Introduction
Ant–plant interactions have been intensively investigated during the last three decades and many experimental studies have demonstrated from facultative to obligate mutualistic associations (Janzen 1967; Bentley 1977; Beattie 1985). Ants are by far the most frequent visitors to extrafloral nectaries (EFNs), and their relevance as ant-herbivore agents has been shown for several EFN-bearing plants in both temperate and tropical environments (reviewed by Koptur 1992). Herbivore deterrence on plants with EFNs results from the ants’ predatory/aggressive behaviour near the nectar source. Lepidopteran caterpillars exploiting such ant-visited plants have developed an array of mechanisms to overcome ant predation (Janzen 1967; Brower 1984; Koptur 1984; Heads & Lawton 1985; Costa, Oliveira-Filho & Oliveira 1992; Freitas & Oliveira 1992). Some phytophagous insects can also avoid ant attacks on their host plants by secreting sweet appeasing substances, as seen in ant-attended Homoptera and butterfly larvae in the families Lycaenidae and Riodinidae (Malicky 1971; Atsatt 1981; Messina 1981; Pierce & Mead 1981; Fritz 1983; Buckley 1987).

Ant influence on butterfly biology and behaviour is well documented for mutualistic associations involving ants and myrmecophilous larvae. In several instances ants have been demonstrated to affect larval and adult behaviour, larval growth and mortality, and female oviposition (Malicky 1971; Atsatt 1981; Pierce & Mead 1981; Pierce & Elgar 1985; Cottrell 1984; DeVries 1984, 1991; Thomas et al. 1991). For all these cases, the butterflies were shown to benefit from living in proximity to ants. On the other hand, eggs and
larvae from non-mymecophilous butterflies are frequently preyed upon or removed from host plants by foraging ants. A few studies have examined how such negative interference from ants can affect butterfly biology and behaviour; but little experimental data are presented and the vast majority concerns the Heliconini (Benson, Brown & Gilbert 1976; Brown 1981; Turner 1981; Smiley 1985, 1986).

*Eunica bechina* Talbot (Nymphalidae) is a non-mymecophilous butterfly whose larvae feed on *Carayocar brasiliense* Camb. (Caryocaraceae), a plant bearing EFNs on sepal and leaf buds, and which is visited day and night by many nectar-gathering ant species in the Brazilian savannah (Oliveira & Brandão 1991). Eggs are laid singly on young leaves, which the catterpillars find preferable to feed on. First to fourth instar catterpillars construct stick-like frass chains at leaf margins (Fig. 1), on the tip of which they rest (Oliveira & Freitas 1991). While feeding on leaves, catterpillars risk being attacked and killed by foraging ants that are attracted to the host plant by its nectary secretions (Freitas & Oliveira 1992). Ant-excluded shrubs of *Carayocar* are more infested by eggs and larvae of *Eunica* than plants with free ant access (Oliveira & Oliveira-Filho 1991). Field observations indicate that foraging ants do not chase ovipositing butterflies. We carried out a series of field experiments to better understand the selective forces underlying such an ant-butterfly interaction.

We investigated the following questions: (i) Do foraging ants destroy butterfly eggs or remove them from the host plant? (ii) Does ant occupation affect the oviposition behaviour of the butterflies and is this related to ant density? (iii) Do adult butterflies visually avoid ovipositing on ant-occupied plant locations? (iv) Does ant occupation affect the survival of butterfly larvae on host plants and is this related to ant density? (v) Is the vulnerability to ant predation affected by larval size? (vi) Do the frass chains constructed by catterpillars provide an efficient refuge against ant predation on host plants?

**Materials and methods**

Field work was carried out from September to December of 1992 and 1993, in a 'cerrado' savannah near Itirapina (21°15'S, 47°49'W), South-east Brazil. All experiments were performed on paired branches of individual *Carayocar brasiliense* shrubs (35–150 cm tall), to eliminate unknown among-plant differences (such as nutritional quality of leaves or location of the plant) that could affect the results. Branches in a given pair were equal in size, shape and number of leaves, each being assigned by the flip of a coin as either a treatment or control branch. All other branches were clipped off to force adult *Eunica bechina* to choose between experimental branches during oviposition experiments. Clipping apparently did not affect the quality of foliage or EFN secretion on experimental branches. Only young leaves were left on experimental branches because they were preferred by *Eunica* larvae as food and by adult females as oviposition sites (Oliveira & Freitas 1991; Freitas & Oliveira 1992). Ants were impeded from climbing on treatment branches by coating the base of the branch with a sticky barrier of tree Tanglefoot® (Tanglefoot Co., Grand Rapids, Michigan, USA). Tanglefoot was applied to control branches on only one side of the stem, so that ants could still reach the foliage. Grass bridges providing aerial access of ants to either experimental branch were eliminated.

Prior to each experimental series, ant density on *Carayocar* shrubs was evaluated by six censuses, every 20–30 min, between 10.00 and 14.30 h. A plant was considered to have low ant density if it averaged 0.5 or fewer ants per branch (mean ± SD = 0.25 ± 0.20, n = 56), and a high ant density for higher values (mean ± SD = 1.25 ± 1.19, n = 57; see also Smiley 1986). High ant densities (on control branches) were also provoked by applying on leaves 2–3 drops of 50% diluted honey (Rashbrook, Compton & Lawton 1992). The same was done on treatment branches to control for effects of unequal attractiveness to organisms not impeded by the Tanglefoot barrier, such as parasitic and predatory wasps, and ovipositing butterflies. Although plants with drops of diluted honey were more frequently visited by ants, at any given time the range of ant visitors to these plants (0–15 ants; n = 59 plants) was roughly equivalent to that observed naturally (0–20 ants; n = 54 plants). The presence of diluted honey drops on leaves had no apparent effect on ant aggressiveness toward catterpillars (see below).

Egg removal by foraging ants was tested by exposing eggs on highly visited branches for 24 h. We recorded the location of the eggs laid on each branch of the experimental pairs.

Oviposition by *Eunica* on *Carayocar* was tested under low and high ant densities. Prior to each trial all eggs and larvae were removed from both branches of a pair (all plants used in the experiments were previously infested by eggs and/or larvae of *Eunica*). Any additional eggs on control and treatment branches were recorded after 24 h. To test whether ovipositing females would visually avoid ant-occupied locations on *Carayocar* shrubs, we used similar-sized (1 cm) black rubber ants (treatment) and black rubber circles (control) as visual cues to ovipositing *Eunica* females. On each branch of an experimental pair (see above) two objects of the same type were each glued with nail polish on a young leaf (two per branch). A tanglefoot barrier impeded ant access to both branch categories of a shrub. *Eunica* eggs were censused after 24 h.

Ant effects on catterpillar survival was tested under low and high ant densities. Both branches of an experimental pair had equal numbers of similar-sized catterpillars (1st and 2nd instars). For control branches we only used well established larvae (frass chain already
constructed). Ant predation on larvae was measured as the number of larvae removed after 24 h.

Behavioural interactions between Eunica caterpillars and ants were recorded during provoked encounters on Caryocar (see Freitas & Oliveira 1992). Encounters were provoked by removing the caterpillars from their frass chains and placing them on plant branches highly visited by ants. Ant–caterpillar interactions were recorded over a period of 15–30 min.

Trials involved caterpillars in three size classes: (1) <6 mm (1st and 2nd instars); (2) 6–20 mm (3rd and 4th instars); (3) >20 mm (5th instar).

The efficiency of frass chains as a refuge against ants was evaluated by using live workers of the termite *Amitermes euamighatus* Silvestri as baits (see Oliveira, Silva & Martins 1987; Bentley & Benson 1988). Live termites were glued with nail polish by the dorsum, to the end of abandoned frass chains and on leaves of

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**Fig. 1.** (a) Second instar caterpillar of *Eunica bechira* feeding at the margin of a young leaf of *Caryocar brasiliense*, next to its frass chain. (b) At the approach of a *Camponotus* worker, the caterpillar takes refuge on the tip of the frass chain and keeps motionless. The ant eventually walks away. A previously used frass chain is shown upper left.
Ant effects on butterfly behaviour

Fig. 2. Egg-laying by *Eunica bechina* on experimental branch pairs of *Caryocar brasiliense*. Values are means. Medians and ranges are given above and inside the bars, respectively. Mann–Whitney *U*-tests: low ant density *U* = 335-5, *P* = 0-3213; high ant density *U* = 461, *P* = 0-0002.

Fig. 3. Egg-laying by *Eunica bechina* on experimental branch pairs of *Caryocar brasiliense* bearing rubber objects. Values are means. Medians and ranges are given above and inside the bars, respectively. Mann–Whitney *U*-test: *U* = 163-5, *P* = 0-0248.

Fig. 4. Effects of ant visitation to shrubs of *Caryocar brasiliense* on the survival of *Eunica bechina* larvae. *G* tests: low ant density *G* = 2-82, d.f. = 1, *P* = 0-1645; high ant density *G* = 9-17, d.f. = 1, *P* = 0-0062.

Fig. 5. Ant predation on *Eunica bechina* caterpillars of different size classes. Percentage values bearing different letters are significantly different. *χ²* tests between different size classes: 1st–2nd vs. 3rd–4th, *χ²* = 4-77, d.f. = 1, *P* = 0-029; 1st–2nd vs. 5th, *χ²* = 27-53, d.f. = 1, *P* = 0-0001; 3rd–4th vs. 5th, *χ²* = 15-24, d.f. = 1, *P* = 0-0001.

ant-patrolled *Caryocar* plants. Termite removal by foraging ants was monitored simultaneously for both plant locations over a period of 10 min.

Results

The removal of *E. bechina* eggs was not affected by ant presence on the host plants; two eggs were removed on branches with ants vs. three on branches without ants (*G* = 0-22, *P* = 0-639, *n* = 28 branch pairs, d.f. = 1). The oviposition behaviour of *E. bechina* females was negatively affected by ant presence, but the effect was significant only under high levels of ant visitation (Fig. 2). The experiments with rubber ants suggest that the butterflies visually avoid ovipositing on plant locations with ants (Fig. 3). Larval mortality was strongly affected by the level of ant visitation to the host plants (Fig. 4), and vulnerability to ant predation decreased with larval size (Fig. 5). The frass chains constructed by the caterpillars were shown to be an effective refuge against ant predation (see Fig. 1). The proportion of live termites attacked by foraging ants on leaves greatly surpassed that recorded for frass chains (Fig. 6).

Discussion

The inhibitory effect of ants on the oviposition by herbivores on ant-occupied plants may result either from the ants’ aggressive behaviour towards ovipositing females (Janzen 1967; Inouye & Taylor 1979; Schenske 1980) or from egg removal by the ants (e.g. Letourneau 1983). Oviposition by *E. bechina* females lasts only 1–3 s and ants were never observed chasing them off the host plant. Therefore, the difference in oviposition rates by *E. bechina* between treatment and control branches is probably due to the discrimination abilities of ovipositing females. It is known that butterflies use visual cues to evaluate plant quality prior to oviposition (Rausher 1978, 1979; Williams & Gilbert 1981). Although the influence of ants on the oviposition decisions of butterflies is well documented for myrmecophilous rosinids and lycaenids (e.g. Atsatt
Fig. 6. Ant predation on live termites placed on leaves of *Caryocar brasiicense* and on frass chains constructed by *Eunica bechinha* caterpillars. $G$ test: $G = 22.18$, d.f. = 1, $P = 0.0001$.

1981; Pierce & Elgar 1985), the cues eliciting the ovi-position response have never been determined. To our knowledge this is the first demonstration that ant presence on the host plant can significantly affect egg-laying by a non-mycmecophilous butterfly. Our finding is relevant in view of the fact that egg-laying decisions by female butterflies are under the influence of an environmental feature (presence of ants) rather than a trait of the host plant (such as the egg-mimics in the Passifloraceae; see Williams & Gilbert 1981) or the herbivore itself (such as the red-eggs of some pierid butterflies; see Shapiro 1981). Although ant movements and chemical cues cannot be dismissed from affecting the choice of *E. bechinha* females, our data demonstrate that ant presence per se can be enough to produce an avoidance response.

Ant predation on the host plant can be a major mortality source for non-mycmecophilous butterfly larvae on ant-visited plants, especially during the establishment phase (Smiley 1998). Our results show that such predation pressure depends both on the level of ant visitation to the host plant, as well as on the size of the caterpillar (see also Tilman 1975; Smiley 1986). Frass chains were demonstrated to be an effective refuge against ant predation, and we suggest that larval vulnerability to ant predation might be critical after egg hatching or during the construction of a new chain. Caterpillars remain on their chains while feeding at leaf margins and assume a motionless posture on the tip of the chain at the approach of an ant (Fig. 1). Ants walk away after a few attempts to reach the larvae and keeping motionless is probably important for discouraging the ants. We observed that the excitement of live termites on the frass chains occasionally elicited further attempts by attacking ants, which ended up destroying the chain and reaching the prey.

According to Schemske (1980), ants provide a consistent defence system, relatively immune to evolutionary changes by the herbivore. Heads & Lawton (1985), however, suggest that herbivores inhabiting ant-visited plants must develop mechanisms to decrease the risk of ant predation. *Eunica bechinha* possesses several of these mechanisms, both as larva and adult (see also Freitas & Oliveira 1992). We suggest that the discriminatory abilities of *E. bechinha* females are finely linked with the risk of ant predation to caterpillars. Although frass chains might not necessarily have evolved as a response to predation risk on anti-visited host plants (they occur widely in the Nymphalidae; see DeVries 1987), this study shows that this behavioural trait helps *E. bechinha* to utilize a plant that is frequently occupied by ants.

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