

SHORT COMMUNICATION

# Larval defence against ant predation in the butterfly *Smyrna blomfieldia*

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## Introduction

Predator avoidance strategies of lepidopteran larvae include morphological traits, such as scoli, spines, and hairs (Frost, 1959), chemical defence, such as unpalatability (Brower, 1984), camouflage and mimicry (Edmunds, 1974), myrmecophily (Malicky, 1970; DeVries, 1991), and behavioural traits, including gregariousness, leaf folding and rolling, mining, regurgitating, suspending by silken threads, hiding, resting on island-like leaf segments, and constructing frass chains (Heads & Lawton, 1985; Costa *et al.*, 1992; Freitas & Oliveira, 1992, 1996; Stamp & Casey, 1993; Loeffler, 1996; Salazar & Whitman, in press).

Frass chains are stick-like structures formed by fecula and silk where the larvae rest when not feeding, which provide an efficient refuge against walking arthropod predators by isolating the larvae from the leaf blade when not feeding (Freitas & Oliveira, 1996). The construction of frass chains by early-instar larvae is a widespread behaviour among the nymphalid butterflies in the subfamilies Eurytelinae, Charaxinae, Limenitidinae, and the tribe Coloburini (Nymphalinae) (Muyshondt, 1973a,b,c, 1974, 1976; Muyshondt & Muyshondt, 1976, 1978; Casagrande & Mielke, 1985; DeVries, 1987; Aiello, 1991; Freitas & Oliveira, 1992). Although Freitas and Oliveira (1996) suggested that frass chains might not necessarily have evolved as a response to predation on ant-visited host plants, the behaviour of resting or taking refuge on these structures diminished larval predation or removal by ants.

*Smyrna blomfieldia* Fabricius (Nymphalidae: Coloburini) is a non-myrmecophilous butterfly, the larvae of which feed on *Urera baccifera* (L.) Gaudich. (Urticaceae), a nettle found commonly in secondary environments such as forest edges and gaps, especially in shaded places. Females lay eggs on many parts of the plant including shoot tips, mature and new leaves, and even spines on the stem near the apical meristem. First (up to 5 mm) to mid third (up to 20 mm) instars construct frass chains, on the tip of which they rest when not feeding.

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The study reported here was designed to provide data on larval behaviour and its relation to ant predation on the host plant, and to evaluate through field experiments the efficiency of frass chains as refuges against walking predators.

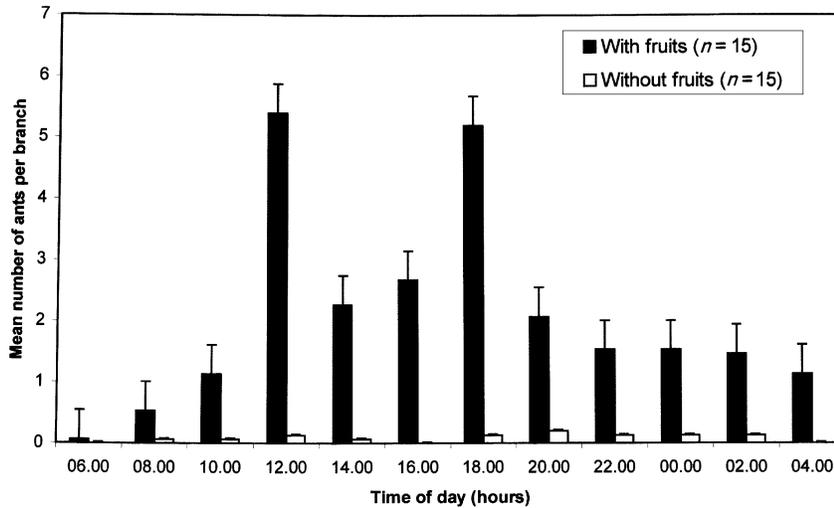
## Materials and methods

Field work was carried out from April to September 1999, and April to August 2000 in the 250-ha semi-deciduous forest Reserva da Mata de Santa Genebra (22°49'S, 47°06'W), Campinas, São Paulo, SE Brazil. The climate of the region is characterised by a dry, cold season (May to August) and a wet, warm season (September to April).

The experiments were performed on branches of *Urera baccifera* shrubs (60–200 cm tall) located along a 1100-m trail within the forest. Ants were prevented from climbing on treatment branches by coating the base of the branch with a sticky barrier of Tree Tanglefoot® (Tanglefoot Co., Grand Rapids, Michigan). Tanglefoot was applied to control branches on only one side of the stem, so ants could still reach the foliage. Grass stems that might have provided ants with access to any experimental branch were eliminated.

Survival of larvae of *S. blomfieldia* discovered on their host plants, and the efficiency of frass chains as a refuge against ants, were evaluated through two field experiments. In the first, to assess ant predation on larvae, 46 larvae (23 on control and 23 on treatment branches) on 31 branches were monitored from May to June 1999, and ant predation was measured as the number of larvae disappearing after 4 days. All larvae found on the *Urera* shrubs along the trail on day 1 ( $n = 46$ ) were used in this experiment. Only two of the shrubs were fruiting.

In the second experiment, 75 live workers of the termite *Armitermes euamignathus* Silvestri were glued using water soluble paste by the dorsum to three different places on ant-visited plants (as in Oliveira *et al.*, 1987; Bentley & Benson, 1988; Freitas & Oliveira, 1996): at the end of abandoned frass chains, on leaves, and on the branches. Each plant location received one termite. Twenty-five different plants were used for each experimental group, six with fruits, 19 without fruits. Termite removal by foraging ants was monitored for 1 h.



**Fig. 1.** Ant visitation throughout the day on shrubs of *Urera baccifera* with and without fruits (*Atta* and *Acromyrmex* excluded). The censuses were carried out on 15 and 16 May 1999 in the Mata da Reserva de Santa Genebra, Campinas, Brazil.

A third experiment was carried out to test whether herbivores on plants with fruits are more likely to be attacked than herbivores on plants without fruits. In this experiment, live termite workers were glued by the dorsum on the leaves (near the petiole) of 84 plants with fruits and 77 plants without fruits. Each plant received one termite, and termite removal by foraging ants was monitored for 1 h.

To determine the species of ants visiting branches of *U. baccifera*, specimens were collected throughout the day (26 h total) and night (8 h total), and fixed in 70% ethanol for later identification. All but two species found (*Atta sexdens* and *Acromyrmex* sp.) were observed as carnivorous in field work (G. Machado and A. V. L. Freitas, pers. obs.). Thirty branches on different shrubs of *U. baccifera* were tagged (15 bearing fruits, 15 without fruits), and were censused every 2 h for 24 h to verify whether there was differential attraction to plants with and without fruits. During each census, the individual branches were searched for ants for 40 s and the number of individuals of each ant species was recorded.

The number of plants with and without fruits was estimated along the trail, and changed from near 1:1 in April/June to a complete disappearance of fruits in September in both years (G. Machado and A. V. L. Freitas, pers. obs.).

## Results and discussion

After 4 days, 60.9% of *Smyrna blomfieldia* larvae had disappeared from the control branches (ants present) while only 17.4% had disappeared from the treatment branches (ants excluded) ( $\chi^2 = 9.14$ , d.f. = 1,  $P < 0.01$ ), suggesting that ant presence could be one of the main factors affecting larval survival. No attacks on live termites on the frass chains were recorded, and the proportion of termites attacked by foraging ants on leaves and branches was 20 and 25% respectively. Termite baits were significantly more removed from the

foliage (leaves and branches) than from the frass chains (two-tailed Fisher exact test,  $P = 0.025$ ). Foraging ants took significantly more termites on plants with fruits (27.4%) than on plants without fruits (14.3%) ( $\chi^2 = 4.14$ , d.f. = 1,  $P < 0.05$ ), suggesting that larval survival on plants with fruits can be reduced strongly as a consequence of high ant-visitation rates.

The ant species recorded on *U. baccifera* branches and their occurrence throughout the day are shown in Table 1. Ants visited mainly shrubs bearing fruits (Mann-Whitney  $U = 22\,164$ ,  $P < 0.001$ ) both during the day and at night (Fig. 1). This difference was also significant if the Attini leaf-cutter ants *Atta* and *Acromyrmex* were not considered (Mann-Whitney  $U = 21\,961$ ,  $P < 0.001$ ). These two species of non-carnivorous ants were found on only one plant censused and were never observed cutting leaves of *U. baccifera*.

Ants can remove the entire diaspore (*Atta sexdens*, *Acromyrmex* sp.) or eat the pulp *in situ* (all other species). Several ant species also walk on the leaves in a typical patrolling behaviour; one *Camponotus* ant was seen preying on a second-instar larva. In provoked encounters, larvae of *Smyrna* may present a series of behaviours considered defensive (see Heads & Lawton, 1985), including regurgitation, wriggling vigorously (beat reflex), and dropping off the plant, hanging by silken threads. These behaviour patterns are very common in larvae of most butterfly species, except the Lycaenidae (Malicky, 1970).

Ant predation can be the major source of mortality for non-mymecophilous butterfly larvae on ant-visited plants (Freitas & Oliveira, 1996), especially during the establishment phase (*sensu* Smiley, 1985). Several behavioural traits can overcome this pressure, however, including frass chains constructed by caterpillars. This structure was shown to be an effective refuge against ant predation in the nymphalid *Eunica bechina* Talbot, which feeds on *Caryocar brasiliensis* Camb. (Freitas & Oliveira, 1996), a plant with extrafloral nectaries. The number of *S. blomfieldia* larvae disappearing from ant-isolated branches

**Table 1.** Ant fauna visiting *Urera baccifera* shrubs at Reserva da Mata de Santa Genebra, Brazil. \*Indicates ant species that do not attack herbivores; +, ant species present; –, ant species absent.

Ant species	Period		Seen	
	Day	Night	Visiting fruit	Attacking baits
<b>Formicinae</b>				
<i>Camponotus abdominalis</i>	–	+	+	+
<i>Camponotus</i> cf. <i>lespesi</i>	–	+	+	+
<i>Camponotus crassus</i>	+	–	–	+
<i>Camponotus sericeiventris</i>	+	–	–	+
<i>Camponotus</i> sp. 1	+	–	–	–
<i>Camponotus</i> sp. 2	+	–	–	–
<i>Paratrechina</i> sp.	+	–	–	+
<b>Myrmicinae</b>				
<i>Acromyrmex</i> sp.*	+	–	+	–
<i>Atta sexdens</i> *	+	–	+	–
<i>Cephalotes pusillus</i>	+	–	–	–
<i>Crematogaster</i> sp.	+	–	+	+
<i>Pheidole</i> sp. 1	+	–	+	+
<i>Pheidole</i> sp. 2	+	–	–	–
<i>Solenopsis</i> sp.	+	–	–	+
<b>Ponerinae</b>				
<i>Pachycondyla villosa</i>	+	–	–	+
<b>Pseudomyrmicinae</b>				
<i>Pseudomyrmex</i> sp.	+	–	–	+

of *U. baccifera* was significantly lower than in ant-visited branches, supporting the idea of frass chains as a larval refuge against walking predators (Freitas & Oliveira, 1992). Although the number of larvae disappearing in ant-visited branches is high (near 60%), these data should be interpreted with care. Some of these individuals might not have been preyed on and may represent larvae moving to neighbouring plants. Nevertheless, they demonstrate that fruits can increase ant visitation to *U. baccifera*, analogous to the attractiveness of extrafloral nectaries in other plants, so the frass chains of *S. blomfieldia* may be a particularly important anti-predator refuge during the fruiting period.

Although the proportions of *U. baccifera* with and without fruits in the field were approximately equal in April/June, most of the larvae found in the field (44 of 46) were feeding on plants without fruits (Yates corrected  $\chi^2=21.9$ , d.f.=1,  $P<0.001$ ). The greater percentage of larvae on shrubs without fruits may result from the ants' aggressive behaviour towards ovipositing females (see Janzen, 1967; Inouye & Taylor, 1979; Schemske, 1980), female choice of host plants (Freitas & Oliveira, 1996), egg removal (Letourneau, 1983), or larval predation on plants with high ant visitation. In *E. bechina*, adult females avoid laying eggs on highly visited branches, and visual cues have been shown to mediate the choice of oviposition in the host plant (Freitas & Oliveira, 1996). Thus, it is possible that females of *S. blomfieldia* may use the fruits as cues of high ant visitation and avoid oviposition on these plants. Experiments are needed to assess whether

differences in host plant use in the field occur due to female selection or differential predation by ants.

The results presented here reinforce the idea that, even if frass chains might not have evolved as a direct response to the risk of ant predation, they increase larval survival on plants that attract ants through extrafloral nectaries (Freitas & Oliveira, 1996) or when fruiting (this study).

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