

Exploitation of lycaenid-ant mutualisms by braconid parasitoids

Konrad Fiedler¹, Peter Seufert¹, Naomi E. Pierce², John G. Pearson³ and Hans-Thomas Baumgarten¹

¹ Theodor-Boveri-Zentrum für Biowissenschaften, Lehrstuhl Zoologie II, Universität Würzburg, Am Hubland, D-97074 Würzburg, Germany

² Museum of Comparative Zoology, Harvard University, 26 Oxford Street, Cambridge, MA 02138-2902, USA

³ Division of Natural Sciences and Mathematics, Western State College, Gunnison, CO 81230, USA

Abstract. Larvae of 17 Lycaenidae butterfly species from Europe, North America, South East Asia and Australia were observed to retain at least some of their adaptations related to myrmecophily even after parasitic braconid larvae have emerged from them. The myrmecophilous glandular organs and vibratory muscles of such larval carcasses remain functional for up to 8 days. The cuticle of lycaenid larvae contains extractable "adoption substances" which elicit antennal drumming in their tending ants. These adoption substances, as well, appear to persist in a functional state beyond parasitoid emergence, and the larval carcasses are hence tended much like healthy caterpillars. In all examples, the braconids may receive selective advantages through myrmecophily of their host larvae, instead of being suppressed by the ant guard. Interactions where parasitoids exploit the ant-mutualism of their lycaenid hosts have as yet been recorded only from the *Apanteles* group in the Braconidae-Microgasterinae.

KEY WORDS: Lycaenidae, Formicidae, myrmecophily, adoption substances, parasitoids, Braconidae, *Apanteles*, defensive mechanisms

INTRODUCTION

Parasitoid wasps or flies are major enemies of the early stages of most Lepidoptera (Shaw 1990, Weseloh 1993). The mostly endophagous larvae of the wasp family Braconidae usually develop in caterpillars of various Lepidoptera or, more rarely, in the larvae of certain Hymenoptera or Diptera. Larvae of Microgasterinae braconids are either solitary or gregarious parasitoids, depending on the species (Papp 1990, Shaw 1990). In this economically important subfamily, the parasitoid larvae leave their hosts and pupate externally in a silken cocoon. This cocoon may either be attached to the host's carcass, as in the case of the well-known *Cotesia glomerata* (L.) parasitizing the cabbage white, *Pieris brassicae* (L.), or to the hostplant. With very few exceptions (e.g. Brodeur and Vet 1994), a host caterpillar will die soon after Microgasterinae larvae have emerged.

Paper submitted 16 December 1994; revised manuscript accepted 29 March 1995.

Lepidopterous caterpillars have evolved a variety of strategies to escape parasitoid attacks. One peculiar strategy is found among myrmecophilous members of the butterfly families Lycaenidae and Riodinidae. These larvae attract ants with the help of specialized exocrine glands (Malicky 1969, Pierce 1983, Cottrell 1984). Larval secretions contain carbohydrates and amino acids which serve as additional nutrition for tending ants (Maschwitz et al. 1975, Pierce 1983, Fiedler & Maschwitz 1988a). In turn, the ants may effectively protect the caterpillars against certain enemies, including parasitoids (Pierce & Mead 1981, Pierce & Easteal 1986, Pierce et al. 1987, Seufert & Fiedler 1994). Such butterfly-ant interactions then represent true mutualisms, analogous to the well-known trophobiotic associations between honeydew-producing Homoptera and ants.

At least three types of myrmecophilous glands are important in lycaenid-ant interactions. The dorsal nectar organ (located mediodorsally on the 7th abdominal segment, DNO hereafter) secretes droplets of a nutrient-rich fluid when stimulated through antennation by ants (Malicky 1969, Cottrell 1984). In addition, many Lycaenidae caterpillars possess a pair of eversible tentacle organs (TOs hereafter) on the 8th abdominal segment. These organs are mostly everted when the caterpillars are disturbed, or while moving to feeding or resting places. Ants respond with a state of alert to TO eversions, apparently mediated through volatile chemicals (Henning 1983, Fiedler & Maschwitz 1988b, Ballmer & Pratt 1992). A third type of myrmecophilous organs are the pore cupolas (PCOs), minute hair-derived epidermal glands whose secretions are often highly attractive to ants (Malicky 1969, Pierce 1983). PCOs are generally found in larvae as well as pupae of lycaenid butterflies, even if stable symbiotic associations with ants do not occur. Finally, so-called dendritic setae appear to be related to caterpillar-ant interactions, since the locations of these setae in larvae or pupae generally receive the greatest attention of visiting ants (Ballmer & Pratt 1992, Fiedler, pers. observ.).

In addition, immatures of many Lycaenidae species produce substrate-borne vibrations (DeVries 1991a). In analogy to the "calls" of certain myrmecophilous Riodinidae (DeVries 1990), vibrations of Lycaenidae larvae may enhance their symbioses with ants, although the occurrence of substrate-borne vibrations in certain non-myrmecophilous lycaenid species suggests that vibratory behavior is not exclusively connected with myrmecophily and may serve another function (possibly defense) in these species (Schurian & Fiedler 1991, Fiedler 1992a, 1994; see also Downey & Allyn 1978 for pupal sounds).

Behavioral interactions between lycaenid caterpillars, their parasitoids and attendant ants have as yet received little attention, although the protective role of tending ants against parasitoids has been established in a few lycaenid species (Pierce & Mead 1981, Pierce & Easteal 1986, Pierce et al. 1987). Pierce et al. (1987) and Nash (1989) obtained evidence that a specialist parasitoid of the Australian obligate myrmecophile *Jalmenus evagoras* Domovian may use attendant *Iridomyrmex*

anceps ants as host-location cues. Recently, Schurian et al. (1993) described how adult braconid wasps utilize ant-related secretions of their host caterpillars. In this paper, we investigate two additional aspects of such multi-species interactions. First, we use a simple behavioral bioassay to investigate the chemical nature of “ant adoption” substances secreted by larvae of the Nearctic species, *Glaucopsyche lygdamus* Doubleday. Second, we document that particular species of parasitoids consume their lycaenid hosts in ways that take advantage of the myrmecophilous properties of the caterpillars. We here summarize our findings on 17 butterfly species, representing 13 genera in 2 subfamilies.

MATERIALS AND METHODS

Adoption substances in *Glaucopsyche lygdamus* larvae

100 final instar caterpillars of the Nearctic *G. lygdamus* were sampled at Gothic, Colorado (elevation 2900 m), in July 1980 and stored frozen at -20 °C. From these larvae, two groups of tissue preparations were made, viz. “dorsal epidermis” and “ventral epidermis”. PCOs as well as other setae which may play a role in ant-caterpillar interactions (e.g. dendritic setae: Ballmer & Pratt 1992) are too small to permit individual excision, but morphological analyses revealed that these structures are common dorsally, but rare (PCOs) or absent (dendritic setae) on the ventral side of the caterpillars (e.g. Ballmer & Pratt 1989). Wet tissue samples (208 mg dorsal epidermis and 20 mg ventral epidermis) were weighed and extracted with 50 μ l of solvent per mg of tissue. This approach equalizes concentrations of ions or extractable substances between experimental (dorsal) and control (ventral) tissues. Extraction was accomplished by grinding tissue samples in glass vials with flanged glass rods. Redistilled dichloromethane was used as solvent.

Pre-packaged silica gel thin-layer plates (EM, 25 μ) were spotted with 100 μ l of tissue extract (dorsal, ventral) or solvent. On some plates cholesterol was also spotted as a standard indicator. Before solvent development, spotted material was first assayed for biological activity with a tissue paper overlay protecting the plates. Plates were then developed at 4 °C with hexane/ethyl-acetate/ethanol (92:6:2), and as soon as these plates had dried, the bioassay was carried out with a tissue paper overlay marked in 1 cm bands for each sample. Following bioassay, separated components were visualized by iodine vapor. Eight trials were conducted.

Queenright colonies of *Formica altipetens*, kept in artificial nests and fed on a diet of honey water and freshly killed insects with access to ad libitum water, were used for bioassays. Treated TLC plates were offered at a distance of 10 cm from the entrance to the ant nest in a foraging arena (71 \times 142 cm) in which an ant colony was placed. Behavioral responses of the ants were scored as a percentage of the number of times that workers stopped and drummed over the total number of encounters during a 15 min period.

Interactions between ants and parasitized caterpillars or larval carcasses

During our studies on the life-cycles of various Lycaenidae species in Central Europe, North America, South East Asia and Australia, we repeatedly collected caterpillars that later turned out to be parasitized. Field-collected caterpillars

of 17 species (Table 1) were reared in the laboratory together with their attendant ants, until parasitoids left their host caterpillars to pupate. Some individuals were left under natural conditions on their hostplants. Behaviors of ants before and after parasitoid emergence were noted, and the activity of the myrmecophilous organs of the caterpillars as well as their ability to produce substrate-borne vibratory signals were followed until the carcasses eventually lost attractiveness to ants. Vibrations were monitored using a stethoscope (Schurian & Fiedler 1991).

RESULTS

Adoption substances in the epidermis of *Glaucopsyche lygdamus*

Whenever worker ants tend lycaenid larvae in nature, a characteristic antennal drumming is one main component of ant-caterpillar interactions (Malicky 1969, 1970). We used this behavioral trait as an indicator of ant response to lycaenid adoption substances: immediately upon encountering a spot of dorsal skin extract, a *F. altipetens* worker would often drum on the spot in exactly the same manner as she would on a caterpillar in the field. Workers never recruited nestmates to the spots, and tactile stimulation appeared to be necessary to elicit ant response as workers did not move preferentially upwind toward fresh caterpillars when air was passed over them in a Y-tube olfactometer (Pierce, unpubl.).

Workers investigated the extract of dorsal skin significantly more often than the extract of ventral skin (i.e. "controls"). With dorsal extracts, $57.0 \pm 17.7\%$ (mean \pm S.D.) of all encounters resulted in drumming responses, whereas with ventral extracts the average figure was $30.6 \pm 17.6\%$ (8 paired trials, $p < 0.005$, Wilcoxon signed-ranks test). Although myrmecophilous organs are almost absent from the ventral surface of a caterpillar, the ventral control extracts still contained low activity. We attribute this to the unavoidable crudeness of the tissue preparation. Fig. 1 presents the data compiled from developed TLC plates. Nine spots were found on each chromatogram for both the dorsal and ventral extracts. These correspond to R_f values of 0.15, 0.18, 0.33, 0.43, 0.45, 0.49, 0.53, 0.54, and 0.96. For each trial, the mobilities and spot sizes were identical between the two samples, while the solvent control did not afford any visualizable material nor did it receive antennation by ants.

The most active band on the TLC ($R_f = 0.96$, section 12) appears near the solvent front. Together with extraction by methylene chloride, this mobility suggests a substance of low polarity. In addition, since the substance remains on TLC plates after initial bioassay, plate development, solvent evaporation and final bioassay, it would seem to be of low volatility. Although we did not quantify this effect, the activity of the substance appeared to decrease with time, suggesting that it eventually evaporates or undergoes chemical alteration on TLC plates. The activity around position 3.5 corresponds to an R_f value of about 0.43, coinciding

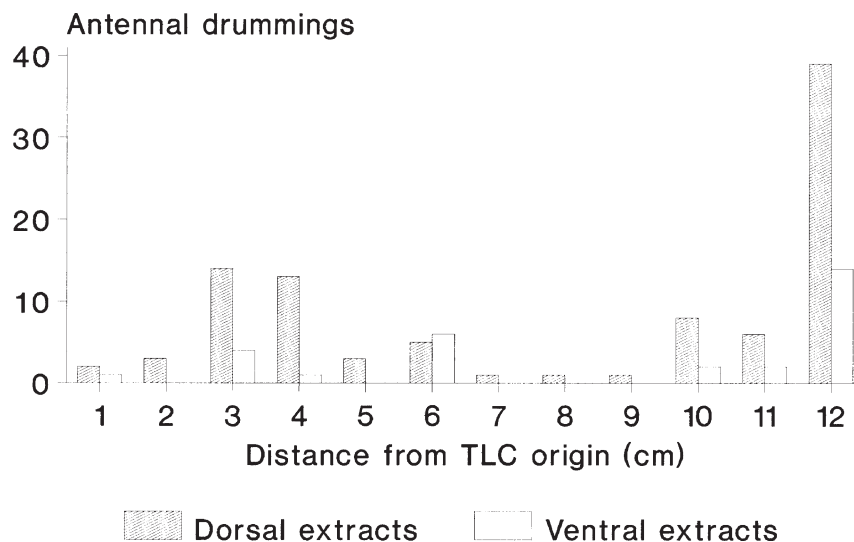


Fig. 1. Total number of antennal drumming responses of *Formica altipetens* worker ants towards separated compounds of epidermal extracts of *Glaucopsyche lygdamus* caterpillars on developed TLC plates. Given are cumulative numbers over a total test period of 120 min. A compound which occurred in section 12 elicited the greatest response and corresponded to an R_f value of 0.96. A compound which occurred inbetween fractions 3 and 4 showed some activity and corresponded to an R_f value of 0.43.

with the respective value of cholesterol. We have no evidence whether the ants are attracted to cholesterol or another compound at that position.

Persistence of myrmecophily in parasitized caterpillars or larval carcasses

In most cases, a parasitized lycaenid caterpillar would die, or has already been killed, when its parasitoid larvae are ready to pupate. This was invariably the case with caterpillars parasitized by Tachinidae flies (observations with following lycaenid species: *Jalmenus evagoras*, *Thecla betulae*, *Arhopala amphimuta*, *Drupadia theda*, *Hypolycaena erylus*, *Rapala dieneces*, *Callophrys rubi*, *Jamides malaccanus*, *J. caeruleus*, *Glaucopsyche alexis*, *G. lygdamus*, *Polyommatus coridon*, *P. icarus*), and with certain ichneumonids (*Hyposoter*, Campopleginae) or braconids (*Aleiodes*, Rogadinae), which pupate inside the host cuticle (e.g. in *Drupadia ravindra*, *Scolitantides orion*, *Aricia eumedon*, *Polyommatus coridon*; Fiedler, pers. observ.). However, when caterpillars are parasitized by members of the *Apanteles* group (Braconidae-Microgasterinae), the larvae often remain attractive to their tending ants and the myrmecophilous organs may stay functional for several days beyond parasitoid emergence. The following observations were made (categorized by the

Table 1. Summary of observations on the function of myrmecophilous organs and the persistence of vibratory abilities in parasitized caterpillars of 17 Lycaenidae butterfly species. Only observations involving parasitoids of the braconid subfamily Microgasterinae are considered. ¹: DNO intensively antennated, but secretion act not observed. ²: not recorded. ³: species with non-functional rudimentary DNO. Facultative myrmecophiles associate with a variety of ant taxa, but are not dependent on ant-attendance. Obligate myrmecophiles invariably live in symbiosis with a specific host ant. A (g) or (s) behind the parasitoid's name indicates gregarious (multiple wasps per host) or solitary parasitoids. The 4 parasitoids from the Apanteles ater-group represent different species.

Butterfly species	Interaction with ants	DNO —— function persisting ——	TOs	Vibrations —— to ants	Attractive Parasitoid
Curetis sp. (n = 3)	not myrmecophilous	absent	3-4 d	3-4 d	Apanteles nr. ater (g)
Jalmenus evagoras	obligate myrmecophile	? ²	? ²	? ²	Apanteles sp.(s)
Surendra florimel (n = 6)	facultative myrmecophile	? ¹	> 4 d	4-7 d	Apanteles nr. ater (s)
Drupadia theda (n = 17)	obligate myrmecophile	? ¹	4-7 d	3-8 d	Apanteles nr. prosymna (g)
Cheritra freja (n = 2)	not myrmecophilous	absent	absent	no	Apanteles nr. prosymna (g)
Hypolycaena othona (n = 1)	facultative myrmecophile	no	absent	6 d	Apanteles sp. (s)
H. erylus	obligate myrmecophile	? ²	? ²	? ²	Apanteles sp.
Callophrys rubi (n = 10)	not myrmecophilous	absent ³	absent	> 2 d	Distatrix sancus (g)
Antheus emolus (n = 14)	obligate myrmecophile	1-3 d	no	2-5 d	Apanteles nr. ater (s)

Jamides malaccanus (n = 4)	facultative myrmecophile	? ¹	1 d	no	yes	Apanteles nr. ater (s)
Caleta manovus (n = 1)	not myrmecophilous	absent	absent	1 d	no	Apanteles sp. (s)
Glaucoptysche alexis (n = 4)	facultative myrmecophile	? ²	? ²	> 2 d	? ²	Cotesia cupreus (g)
G. lygdamus	facultative myrmecophile	? 2	? 2	? 2	yes	Apanteles cyaniridis (g)
Plebejus melissa (n = 1)	facultative myrmecophile	? 2	? 2	? 2	yes	Apanteles sp. (g)
Polyommatus coridon (n = 3)	facultative myrmecophile	? ¹	2 d	2-3 d	yes	Cotesia cupreus (g)
P. coridon (n = 60)	facultative myrmecophile	no	no	no	yes	Cotesia saltatoria (s)
P. bellargus (n = 1)	facultative myrmecophile	6 d	6 d	6 d	yes	Cotesia cupreus (g)
P. icarus (n = 1)	facultative myrmecophile	5 d	6 d	5 d	yes	Cotesia cupreus (g)

myrmecophilous organs, respectively; see Table 1 for synopsis of observations):

THE DORSAL NECTAR ORGAN (DNO) REMAINS ACTIVE IN PARASITIZED CATERPILLARS

Anthene emolus, a widespread Oriental lycaenid butterfly, is an obligatory myrmecophile which is specifically associated with the aggressive weaver ant, *Oecophylla smaragdina*. Caterpillars of *A. emolus* secrete droplets from the DNO at particularly high rates (200-300 droplets/h: Fiedler & Maschwitz 1989). In April and May 1993, we found a total of 14 parasitized individuals (infested by a solitary parasitoid species of the *Apanteles ater* group) at Ulu Gombak (West Malaysia), on the hostplant tree *Saraca thaipingensis* (Caesalpinaceae). From 6 caterpillars, the wasp larva had already emerged and pupated at the time of collection. Each of these caterpillars sat motionless on the silken braconid cocoon attached to its ventral side. The remaining 8 caterpillars were collected as second to early fourth (= final) instars while feeding, and the braconid larvae emerged later in captivity.

In both the field and the laboratory, all 14 caterpillars remained fully attractive to their specific host ant even after the parasitoids had emerged. One or two *Oe. smaragdina* workers constantly attended and antennated each larval "carcass". These responded with regular eversions of the DNO, and the secretion droplets were frequently visible (Fig. 2). *Oe. smaragdina* ants eagerly harvested every single droplet. The ability to deliver DNO secretions persisted in *A. emolus* caterpillars up to 3 days after the braconid larva had emerged. Attractiveness of the caterpillar carcasses to *Oecophylla* ants persisted 4-5 days, and the adult braconids eclosed after a pupal period of 5-6 days. In *A. emolus*, eversions of the TOs were not seen after parasitoid emergence.

DNO secretions after parasitoid emergence could as yet be ascertained in two additional Lycaenidae species, the Palearctic facultative myrmecophiles *Polyommatus bellargus* and *P. icarus*. One mature *P. bellargus* caterpillar (origin near Würzburg, northern Bavaria), from which 24 *Apanteles* larvae had emerged and pupated at the ventral side, secreted droplets from the DNO up to the sixth day beyond parasite emergence. This caterpillar was caged with *Lasius flavus* ants for only 1-5 minutes

Fig. 2. *Oecophylla smaragdina* worker ants drink a secretion droplet from the dorsal nectar organ of a parasitized *Anthene emolus* caterpillar (Ulu Gombak, West Malaysia). The white *Apanteles* cocoon is attached to the ventral side of the host. Photograph P. Seufert.

Fig. 3. A fourth instar caterpillar of *Curetis* sp. (Ulu Gombak, West Malaysia) with everted tentacle organs after tactile disturbance. Note the dense pillow of *Apanteles* cocoons on the ventral side. Photograph P. Seufert.

Fig. 4. Parasitized carcass of mature caterpillar of *Polyommatus icarus*, offering a nectar droplet to tending *Lasius flavus* ants the day after parasitoid emergence. Photograph K. Fiedler.



per day to prevent the rapid depletion of the DNO reservoir content. By this procedure, 21 DNO secretion droplets were observed in a total observation period of 7.5 minutes spread over 4 days. Using the same test procedure with the same ant species, a mature *P. icarus* caterpillar (origin near Würzburg, northern Bavaria) from which 14 braconid larvae had emerged, delivered 12 DNO secretion droplets in 8 minutes total observation period spread over 5 days (Fig. 4).

We observed antennation at, and eversion of, the DNO in larvae of 4 additional species (Table 1) which were kept together continuously with attendant ants. *S. florimel*, *J. malaccanus*, and *P. coridon* are facultative myrmecophiles whose mature caterpillars are tended by a variety of ant taxa (Fiedler 1991, 1992b and unpubl.), while *D. theda* is obligatorily connected with certain *Crematogaster* species (Maschwitz et al. 1985; Seufert & Fiedler 1994). We suppose that secretions occurred even after parasitoid emergence in these cases, but that the gland reservoirs of the caterpillars had been depleted at the time of observation due to continuous milking by ants.

THE TENTACLE ORGANS (TO) REMAIN ACTIVE IN CATERPILLAR CARCASSES

The TOs of 6 myrmecophilous Lycaenidae species (Table 1) remained fully active for up to 7 d beyond braconid emergence and still elicited the typical behavioral reaction (alert, "excited runs": Fiedler & Maschwitz 1988b) in their tending ants. TO eversions occurred in the following pairs of interacting species: *S. florimel* with *Rhoptromyrmex wroughtonii* (Myrmicinae), *Technomyrmex* and *Iridomyrmex* sp. (Dolichoderinae); *D. theda* with *Crematogaster* sp. (Myrmicinae); *J. malaccanus* with *Camponotus* (subgenus *Tanaemyrmex*) sp. (Formicinae) and *Crematogaster*; and the 3 *Polyommatus* species with *Lasius flavus* (Formicinae).

Caterpillars of the Oriental genus *Curetis* (subfamily Curetinae: Eliot 1990) have highly modified and very large TOs situated in elevated epidermal cylinders. The TOs are thrust rapidly after tactile disturbance of a caterpillar, making visible the long, conspicuous, black-and-white hairs (DeVries et al. 1986; Fiedler et al. 1995). This striking defensive behavior continued in three parasitized *Curetis* caterpillars found at Ulu Gombak in May 1993 for 3-4 d after emergence and pupation of the larvae of a gregarious braconid species (*Apanteles ater* group; Fig. 3). A specific identification of the host butterfly is impossible, because the parasitized larvae have been collected on the same hostplant tree (*Millettia atropurpurea*, Fabaceae) together with early stages of *Curetis bulis* and *C. santana*, whose larvae look almost identical. *Curetis* caterpillars are rarely, if ever tended by ants (DeVries 1984; Fiedler et al. 1995).

LARVAL CARCASSES REMAIN ATTRACTIVE TO THEIR TENDING ANTS

Larval carcasses of 12 species (Table 1) were persistently, or at least temporarily, tended by ants for up to 5 d beyond parasitoid emergence.

Such interactions were observed in the field as well as in captivity, involving ants of the genera *Crematogaster*, *Rhoptromyrmex*, *Lasius*, *Formica*, *Camponotus*, *Oecophylla*, *Dolichoderus*, *Technomyrmex* and *Iridomyrmex*. These ants showed the same antennal drumming towards healthy caterpillars, and antennation was not restricted to the DNO nor to the vicinity of the TOs.

PERSISTENCE OF VIBRATORY BEHAVIOR

The ability to produce substrate-borne vibrations persisted in 11 lycaenid caterpillar species including three non-myrmecophiles (Table 1), sometimes up to 8 d beyond parasitoid emergence. Except in *Cheritra freja*, there was no indication that the vibratory behavior of parasitized caterpillars, and later carcasses, differed in any respect from that of healthy caterpillars. Vibratory behavior was most easily elicited by tactile disturbance of the larvae (e.g. handling with forceps). *Ch. freja* is a myrmecoxenous member of the hairstreak subtribe Cheritriti. In this species, even healthy larvae only occasionally made vibratory calls after tactile disturbance (2 out of 15 in our sample), and in two fourth (= final) instars, which were parasitized by a gregarious *Apanteles* species (nr. *prosymna*), calls were recognized neither before nor after parasitoid emergence.

DISCUSSION

Observations on behavioral interactions between lycaenid caterpillars and their parasitoids are typically chance findings. Experimental work with larger sample sizes is prohibited by the scarcity or crypsis of both hosts and parasitoids. Moreover, the taxonomy of the parasitoids involved is still in a very incomplete stage, especially in the tropics, and information on host ranges or specificity is almost unavailable. Hence, the observations and conclusions presented here are by necessity based on small numbers of sometimes anecdotal observations. Nevertheless, collectively these provide circumstantial evidence that certain parasitoids may take advantage of the mutualistic relationships between their lycaenid host larvae and ants.

Breaking the chemical communication code between lycaenids and ants is an essential facet of parasitoid subterfuge. Besides delivery of nutritive secretions (such as those derived from the DNO), lycaenid caterpillars possess extractable components in their integument which serve as "adoption substances". These epidermal substances induce non-aggressive antennal drumming in the ants when tending lycaenid immatures. Although their chemical composition remains unknown, these substances are of low volatility. Physical contact is necessary to induce caterpillar-ant interactions. The adoption substances retain biological activity for some time, as shown in the experiments with extracts. Furthermore, caterpillar carcasses left by braconid parasitoids, and occasionally even empty pupal cases, remain attractive to

tending ants for hours or days. This strongly suggests that the adoption substances remain functional. The PCOs, ubiquitous glandular structures of lycaenid immatures, are likely to be one source of these adoption signals, although the significance of other organs, such as dendritic setae, remains to be addressed.

In *Anthene emolus*, *Polyommatus bellargus*, *P. icarus*, and probably in four additional species where ants antennated the DNO of the larval carcasses, even the ability to secrete droplets from the DNO persisted, although this property typically ceased first. This may be explained by depletion of the secretion supply in the glandular reservoir. After feeding has stopped, caterpillars cannot replenish their secretion stock. The 12 DNO secretion droplets observed in *P. icarus* after parasitoid emergence, and the 21 droplets in the case of *P. bellargus*, closely match the estimated DNO reservoir content in these species. Using Malicky's (1969) histological data, the total reservoir volume is roughly 0.06 μl in *P. icarus*, which corresponds to c. 15 droplets with an average volume of 0.004 μl (Fiedler & Burghardt, unpubl.). For *P. bellargus*, the respective figure is a DNO volume of 0.131 μl corresponding to 22 droplets of 0.006 μl average size (based on histological and experimental data from the closely related *P. coridon*).

Vibratory abilities and TO activity persisted longer than DNO secretions. Both these behaviors are executed by specialized muscles. In addition, limited capacities to move persisted in the Lycaenidae species listed in Table 1. From these observations we conclude that part of the peripheral musculature (i.e. those muscles necessary for DNO and TO activity, or for producing vibratory calls) is exempted from exploitation by these Microgasterine parasitoids. With the exception of *Anthene emolus*, whose parasitized caterpillars reached only half the size of healthy larvae, the larvae listed in Table 1 did not show retarded growth and attained normal size despite parasitoid infestation.

Sparing of myrmecophilous properties occurred in solitary as well as gregarious species, but all records yet available of such phenomena are from the *Apanteles* group in the braconid subfamily Microgasterinae. We have so far never observed comparable interactions in lycaenid caterpillars parasitized by various species of Tachinidae flies, Ichneumonidae wasps, or braconids from other subfamilies (e.g. Rogadinae). Even among the Microgasterinae, this trait is not universal. *Cotesia saltatoria*, for example, is a solitary parasitoid that emerges when the host caterpillars are still rather small (third instar). In this case, the host soon dies after parasitoid emergence, and neither activity of the myrmecophilous organs nor vibratory behavior could be observed, although the carcasses remained attractive to *Lasius flavus* ants for up to 3 d.

Parasitoids of lepidopterous caterpillars greatly vary in the extent of damage they impose on their hosts. All tachinid flies which we have reared so far from Lycaenidae caterpillars leave only a limp cuticle and

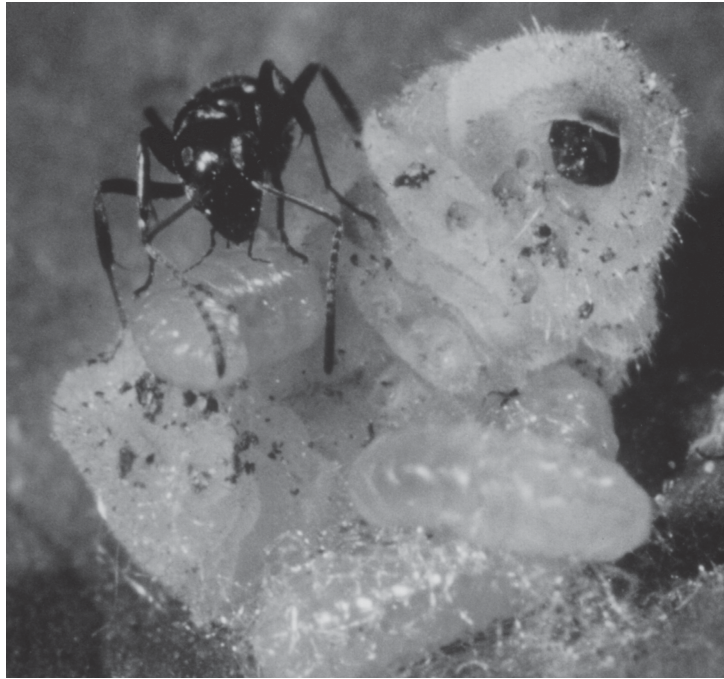


Fig. 5. Larvae of *Apanteles cyaniridis*, freshly emerged from mature *Glaucopsyche lygdamus* caterpillar. The wasp larvae are not attacked by the attendant ant (*Formica* sp.). Photograph N. E. Pierce.

entirely consume all internal host tissues (observations made on a number of *Lycaenidae* species, see above), and neither secretory nor vibratory abilities persisted. Instead, the caterpillars were abandoned by their tending ants 1-2 days prior to parasitoid emergence and vibratory behavior likewise ceased at roughly that time.

If sparing of myrmecophilous properties of *Lycaenidae* hosts should turn out to be a specific adaptation of certain *Microgasterinae* braconids rather than an accidental or commonplace epiphenomenon, what selective advantage may these parasitoids derive from this behavior? Although experimental evidence has not yet been obtained, it is likely that the wasps benefit in at least three ways. First, their pupal cocoons are covered by their well-camouflaged hosts and may thereby escape visually searching predators or hyperparasitoids. In all the species listed in Table 1, except sometimes in *Cotesia saltatoria*, the cocoons are tightly attached to the caterpillar. The gregarious species often formed well-defined pillows of cocoons, on which the host carcass sat motionless for days (Fig. 3).

Second, the potentially vulnerable larvae of braconids emerging from the carcasses of their lycaenid hosts somehow avoid attacks by ants which attend the lycaenids. Larvae of *Apanteles cyaniridis* emerging

from *Glaucopsyche lygdamus*, for example, were observed to be inspected by attendant ants, but not attacked (Fig. 5; also Fiedler 1992b). Within a few minutes after emergence from their hosts, these parasitoid larvae spin protective cocoons and pupate beneath the host's carcass. The mechanism for this striking tolerance remains unknown (see DeVries' (1991b) discussion of appeasement versus ignorance).

Finally, by sparing the myrmecophilous properties of their hosts, these braconids are indirectly attended by ants for at least the first part of their own pupal development. Recent work on aphidiid parasitoids of ant-tended aphids has confirmed that certain parasitoids, rather than their host aphids, may benefit from ant-association (Völkl 1992). The interactions between ants, lycaenid caterpillars and Microgasterinae braconids obviously bear a similar potential. In all cases observed so far, the attractiveness of the larval carcasses ceased before the wasps emerged from their cocoons, allowing the wasps to eclose and fly off in the absence of ants.

Together with the apparent use of tending ants as host-location cues by an Australian *Apanteles* species parasitizing *Jalmenus evagoras* (Pierce et al. 1987, Nash 1989), and with the ability of several Microgasterinae wasp species to feed on the secretions which their hosts provide from the myrmecophilous glands (Schurian et al. 1993), our observations indicate that interactions between ants, lycaenid larvae and their braconid parasitoids are even more complex than previously thought.

Acknowledgements. We are most grateful to J. Papp (Natural History Museum, Budapest), K. van Achterberg (Natural History Museum, Leiden), W. R. M. Mason, and I. Naumann (CSIRO, Canberra) for the identification of Braconidae samples. Information on Tachinidae flies was kindly provided by C. W. Sabrosky (National Museum of Natural History, Washington), H.-P. Tschorsnig (Staatliches Museum für Naturkunde, Stuttgart) and H. Shima (Kyushu University, Fukuoka). Vouchers of parasitoids are deposited in the museum collections under their care. Yong Hoi-Sen and Azarae H. Idris (Universiti Malaya, Kuala Lumpur) kindly supported the field work of K.F. and P.S. at Ulu Gombak in Malaysia. B. Hölldobler provided constructive criticism on an earlier manuscript version. This work was supported by the Deutsche Forschungsgemeinschaft (Fi 547/1-1 and a postdoc fellowship to K.F. from the Leibniz Award to B. Hölldobler).

LITERATURE CITED

- BALLMER, G. R. & PRATT, G. F. 1989. A survey of the last instar larvae of the Lycaenidae of California. *J. Res. Lepid.* 27:1-81.
- . 1992. Quantification of ant attendance (myrmecophily) of lycaenid larvae. *J. Res. Lepid.* 30:95-112.
- COTTRELL, C. B. 1984. Aphytophagy in butterflies: its relationship to myrmecophily. *Zool. J. Linn. Soc.* 79: 1-57.
- BRODEUR, J. & VET, L. E. M. 1994. Usurpation of host behaviour by a parasitic wasp. *Anim. Behav.* 48:187-192.

- DEVRIES, P. J. 1984 Of crazy-ants and Curetinae: are *Curetis* butterflies tended by ants? Zool. J. Linn. Soc. 79:59-66.
- . 1990. Enhancement of symbioses between butterfly caterpillars and ants by vibrational communication. Science 248:1104-1106.
- . 1991a. Call production by myrmecophilous riodinid and lycaenid butterfly caterpillars (Lepidoptera): morphological, acoustical, functional, and evolutionary patterns. Amer. Mus. Novit. 3025:1-23.
- . 1991b. Mutualism between *Thisbe irenea* butterflies and ants, and the role of ant ecology in the evolution of larval-ant associations. Biol. J. Linn. Soc. 43:179-195.
- DEVRIES, P. J., HARVEY, D. J. & KITCHING, I. J. 1986. The ant associated epidermal organs on the larva of the lycaenid butterfly *Curetis regala* Evans. J. Nat. Hist. 20:621-633.
- DOWNEY, J. C. & ALLYN, A. C. 1978. Sounds produced in pupae of Lycaenidae. Bull. Allyn Mus. 48:1-14.
- ELIOT, J. N. 1990. Notes on the genus *Curetis* Hübner (Lepidoptera, Lycaenidae). Tyô to Ga 41:201-225.
- FIEDLER, K. 1991. Systematic, evolutionary, and ecological implications of myrmecophily within the Lycaenidae (Insecta: Lepidoptera: Papilionoidea). Bonner zool. Monogr. 31:1-210.
- . 1992a. Notes on the biology of *Hypolycaena othona* (Lepidoptera: Lycaenidae) in West Malaysia. Nachr. entomol. Ver. Apollo Frankfurt N. F. 13:65-92.
- . 1992b. The life-history of *Surendra florimel* Doherty 1889 (Lepidoptera: Lycaenidae) in West Malaysia. Nachr. entomol. Ver. Apollo Frankfurt N. F. 13:107-135.
- . 1994. The life-history of *Caleta roxus* (Lepidoptera: Lycaenidae). Nachr. entomol. Ver. Apollo Frankfurt N. F. 14:371-384.
- FIEDLER, K. & U. MASCHWITZ. 1988a. Functional analysis of the myrmecophilous relationships between ants (Hymenoptera: Formicidae) and lycaenids (Lepidoptera: Lycaenidae). II. Lycaenid larvae as trophobiotic partners of ants - a quantitative approach. Oecologia 75:204-206.
- . 1988b. Functional analysis of the myrmecophilous relationships between ants (Hymenoptera: Formicidae) and lycaenids (Lepidoptera: Lycaenidae). III. New aspects of the function of the retractile tentacular organs of lycaenid larvae. Zool. Beitr. Berlin N.F. 31:409-416.
- . 1989. The symbiosis between the weaver ant, *Oecophylla smaragdina*, and *Anthene emolus*, an obligate myrmecophilous lycaenid butterfly. J. nat. Hist. 23:833-846.
- FIEDLER, K., SEUFERT, P., MASCHWITZ, U., & AZARAE, I. 1995. Notes on larval biology and pupal morphology of Malaysian *Curetis* butterflies (Lepidoptera: Lycaenidae). Tyô to Ga 45:287-299.
- HENNING, S. F. 1983. Chemical communication between lycaenid larvae (Lepidoptera: Lycaenidae) and ants (Hymenoptera: Formicidae). J. Entomol. Soc. Sth. Afr. 46:341-366.
- MALICKY, H. 1969. Versuch einer Analyse der ökologischen Beziehungen zwischen Lycaeniden (Lepidoptera) und Formiciden (Hymenoptera). Tijdschr. Entomol. 112:213-298.
- . 1970. New aspects on the association between lycaenid larvae (Lycaenidae) and ants (Formicidae, Hymenoptera). J. Lepid. Soc. 24:190-202.

- MASCHWITZ, U., SCHROTH, M., HÄNEL, H. & THO Y. P. 1985. Aspects of the larval biology of myrmecophilous lycaenids from West Malaysia. *Nachr. entomol. Ver. Apollo Frankfurt, N. F.* 6:181-200.
- MASCHWITZ, U., WÜST, M. & SCHURIAN, K. 1975. Bläulingsraupen als Zuckerlieferanten für Ameisen. *Oecologia* 18:17-21.
- NASH, D. R. 1989. Cost-benefit analysis of a mutualism between lycaenid butterflies and ants. Ph. D. thesis, Oxford Univ.
- PAPP, J. 1990. A survey of the European species of *Apanteles* Först. (Hymenoptera, Braconidae: Microgastrinae) XII. Supplement to the key of the *glomeratus*-group. Parasitoid/host list 2. *Ann. Hist.-Nat. Mus. Natl. Hung.* 81:159-203.
- PIERCE, N. E. 1983. The ecology and evolution of symbioses between lycaenid butterflies and ants. PhD Diss., Harvard Univ.
- PIERCE, N. E. & EASTEAL, S. 1986. The selective advantage of attendant ants for the larvae of a lycaenid butterfly, *Glaucopsyche lygdamus*. *J. Anim. Ecol.* 55:451-462.
- PIERCE, N. E. & MEAD, P. S. 1981. Parasitoids as selective agents in the symbiosis between lycaenid butterfly larvae and ants. *Science* 112:1185-1187.
- PIERCE, N. E., KITCHING, R. L., BUCKLEY, R. C., TAYLOR, M. F. J. & BENBOW, K. F. 1987. The costs and benefits of cooperation between the Australian lycaenid butterfly, *Jalmenus evagoras*, and its attendant ants. *Behav. Ecol. Sociobiol.* 21:237-248.
- SCHURIAN, K. G. & FIEDLER, K. 1991. Einfache Methoden zur Schallwahrnehmung bei Bläulings-Larven (Lepidoptera: Lycaenidae). *Entomol. Z.* 101:393-398.
- SCHURIAN, K. G., FIEDLER, K. & MASCHWITZ, U. 1993. Parasitoids exploit secretions of myrmecophilous lycaenid butterfly caterpillars (Lycaenidae). *J. Lepid. Soc.* 47:150-154.
- SEUFERT, P. & FIEDLER, K. 1994. *Drupadia ravindra* and *D. theda* (Lepidoptera: Lycaenidae): contrasting systems of myrmecophily among two closely related butterfly species. In: A. Lenoir, G. Arnold & M. Lepage (eds.) *Les insectes sociaux*, p. 275. Publ. Univ. Paris Nord, Paris.
- SHAW, M. R. 1990. Parasitoids of European butterflies and their study. In: O. Kudrna (ed.) *Butterflies of Europe*, vol. 2, pp. 449-479. Aula Verlag Wiesbaden.
- VÖLKL, W. 1992. Aphids or their parasitoids: who actually benefits from ant-attendance? *J. Anim. Ecol.* 61:273-281.
- WESELOH, R. M. 1993. Potential effects of parasitoids on the evolution of caterpillar foraging behavior. In: N. E. Stamp & T. M. Casey (eds.) *Caterpillars. Ecological and evolutionary constraints on foraging*, pp. 203-223. Chapman & Hall, New York/London.