GENERAL NOTES

Journal of the Lepidopterists' Society
47(2), 1993, 150–154

PARASITOIDS EXPLOIT SECRETIONS OF MYRMECOPHILOUS LYCAENID BUTTERFLY CATERPILLARS (LYCAENIDAE)

Additional key words: *Polyommatus,* Braconidae, Microgasterinae, ant-association, symbiosis.

Mutualistic interactions that are mediated by the delivery of nutritive material (e.g., nectar, pollen) bear the risk of being exploited by species of organisms outside of the mutualism. Numerous ant species, for example, maintain symbioses with plants bearing extrafloral nectaries (EFN's) or with sap-feeding Homoptera excreting honeydew. Specialized insects other than ants secondarily may utilize these energy-rich fluids (e.g., riodinid butterfly caterpillars: DeVries & Baker 1989; adult nepticulid moths: Downes 1968). Adults and larvae of Miletinae lycaenids induce ant-tended homopterans to release honeydew excretions via tactile stimulation (Maschwitz et al. 1988). In these and many more cases, the secondary beneficiaries of ant-plant or ant-homopteran mutualisms are adapted morphologically and/or behaviorally to overcome or avoid ant predation.

Supposedly mutualistic associations with ants also occur in a large number of species of the butterfly family Lycaenidae (e.g., Fiedler 1991). Myrmecophilous lycaenid caterpillars attract ants with the help of particular epidermal glands. One of these glands, the dorsal nectary organ (DNO), secretes carbohydrates and/or amino acids (Maschwitz et al. 1975; Pierce 1983). These nutritive secretions may cause ants to recruit nestmates to the caterpillars (Fiedler & Maschwitz 1989). In turn, the ants may provide protection against certain parasitoids or predators (e.g., Pierce et al. 1987).

DNO secretions usually are released only when ants antennate the immediate vicinity of this gland, which is equipped with specialized mechanosensory setae (Tautz & Fiedler 1992). Insects other than ants have been recorded to feed on DNO secretions only twice, i.e., thrips (Downey 1965) and Miletinae adults (Gilbert 1976). Here we describe a novel type of interaction: parasitoid wasps that feed on DNO secretions of their lycaenid host larvae.

In May 1991, nine caterpillars of *Polyommatus* (*Lysandra*) *coridon* (Poda 1761) were collected in southern France (Dep. Var. Grand Canyon du Verdon) under their hostplant, *Hippocrepis comosa* L. (Fabaceae). The larvae were attended by the Formicinae ant species *Plagiolepis pygmaea* (Latreille 1798). After 10 days in captivity, 8–12 braconid wasps emerged from each of four of the caterpillars. The braconid larvae soon pupated in silken cocoons attached to the cuticle of the dead caterpillars, and eight days later about 40 male and female wasps of an unidentified (and possibly undescribed) *Apanteles* species (subfamily Microgasterinae) eclosed in the morning (J. Papp & E. Haeselbarth pers. comm.; vouchers in coll. Hungarian Natural History Museum, Budapest).

On the following two days, five caterpillars of *Polyommatus* (*Meleageria*) *daphnis* ([Denis & Schiffermüller], 1775) and one pupa of *P.* (*Lysandra*) *bellargus* (Rottemburg, 1775) were offered to the adult parasitoids. Both these lycaenid species are closely related to the original host which was then no longer available as larva. In addition, all these lycaenids share the same hostplants in nature (*Coronilla* and *Hippocrepis* species).

When one large fourth (=final) instar caterpillar of *P. daphnis* was introduced into a plastic vial with 10 *Apanteles* wasps, a female braconid detected the caterpillar within less than 2 min, crawled back and forth on its dorsum and soon found the DNO. The wasp immediately started to antennate the DNO intensively and then repeatedly fed on the secretion droplets produced by the caterpillar. This association persisted for 20 min, when the wasp left the caterpillar without having parasitized it. Within 1 min another female wasp visited the same caterpillar and fed at the DNO, but again left it without ovipositing. A third female wasp at first antennated the DNO and received several

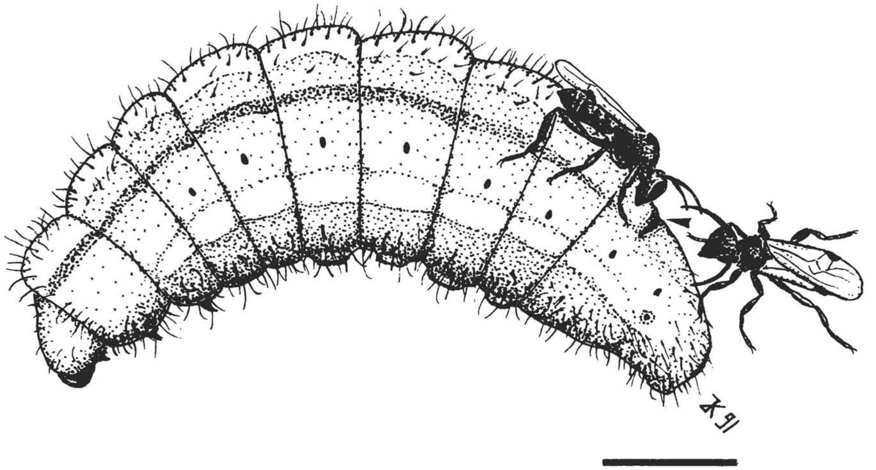

FIG. 1. Two *Apanteles* wasps visiting the DNO (arrow) of a final instar caterpillar of *Polyommatus daphnis*. Scale bar: 2.5 mm. Drawing by J. Klein after a photograph by K. G. Schurian.

secretion droplets, but stung the caterpillar after 6 min and laid one egg. Two further *P. daphnis* caterpillars subsequently were introduced to the same wasps; one was largely ignored while the other was parasitized within 5 min with no further feeding observed at the DNO.

When being stung, the caterpillars abruptly raised their heads and discharged fecal pellets as well as stomodeal regurgitations. However, these defensive behaviors typical of lycaenid caterpillars did not repel the parasitoids.

On the following day, two young fourth instars of *P. daphnis* were offered to the wasps, the latter having been starved for 24 h. Both caterpillars were immediately found by several wasps, and 2–3 braconids constantly elicited the release of carbohydrate secretions by antennating and even vigorously chewing at the DNO (Fig. 1). The wasps also intensively antennated the clusters of pore cupola organs (PCO's) which are situated around the larval spiracles. The PCO's are a second type of lycaenid myrmecophilous organs that are suspected to secrete amino acids (Pierce 1983). Within 1 h, three oviposition acts were observed, all of which were followed by long sequences of feeding at the DNO. During 4 h the caterpillars were continually visited and antennated by 2–3 wasps simultaneously. The wasps now rapidly found their way to the DNO, and few additional oviposition acts were observed.

The wasps were very effective in stimulating secretion acts from the DNO. Within 3 days, with daily observation periods of 1–3 h and about 10 parasitoids per trial, more than 100 secretion acts were observed directly, and all secretion droplets were licked up by the attendant wasps. The caterpillars also sometimes everted their tentacle organs (TO's) which are a third type of myrmecophilous organs in lycaenid larvae. Eversion of the TO's often induces a kind of alertness in attendant ants (Fiedler 1991). There was, however, no detectable effect of the TO's on the braconids. The ability of braconid wasps to release TO eversions is not surprising, since several lycaenid caterpillars also evert these organs when disturbed (Fiedler unpubl.).

Apanteles wasps likewise intensively antennated a pupa of *P. bellargus* and nibbled at it with their mandibles. The wasps' activities mainly concentrated at the pupal abdominal spiracles where dense clusters of the secretory PCO's occur. The wasps apparently harvested the PCO secretions. Larvae of *P. daphnis* were much more attractive to the wasps than was the pupa of *P. bellargus* throughout the trial (1 h).

Female braconids appeared to achieve significant benefits in terms of longevity from their secretion-feeding, while males with their generally short life-span did not. Ten females with no access to lycaenid caterpillars all died within 2 days, whereas 10 females that had fed repeatedly on the DNO secretions survived for at least 3 days. Feeding on homopteran honeydew and flower nectar has been demonstrated to increase both longevity and fecundity in certain braconid wasps (Hagley & Barber 1992). Male braconids were far less attracted to the caterpillars, and despite the presence of numerous male *Apanteles* wasps in the experimental arena, only once did we observe a male feeding on a secretion droplet. All males except this one died within one day.

An ichneumonid wasp, *Agrypon anomelas* (Gravenhorst) (det. H. Schnee), emerged from one of the remaining five *P. coridon* caterpillars. When brought into a vial with 3 fourth instar caterpillars of *P. daphnis*, this ichneumonid neither antennated the larvae intensively nor paid any particular attention to the DNO, despite the occurrence of numerous contacts between the wasp and the caterpillars. Another male ichneumonid randomly captured in the field (*Phygadeuon* sp., a dipteran parasitoid) likewise was unable to facilitate the release of any secretions from the myrmecophilous organs of two *P. daphnis* caterpillars. In both trials the ichneumonids showed little interest in the caterpillars.

In June 1992, we conducted similar trials with two additional Microgasterinae species (collected in the vicinity of Würzburg, northern Bavaria, Germany). *Distatrix sancus* (Nixon 1965) was obtained from field-collected larvae of the myrmecoxenous lycaenid *Callophrys rubi* (Linnaeus 1758). Ten females of this species were caged over night in a petri dish with 2 mature larvae of the highly myrmecophilous lycaenid species *Glaucopsyche alexis* (Poda 1761). Initially, the wasps paid little attention to the caterpillars, but on the following morning both caterpillars were visited by the wasps. Harvesting of DNO secretions occurred often, although the braconid-caterpillar associations were not truly permanent. There were no reactions towards the repeated eversions of the larval TO's, and the wasps did not try to sting the larvae. *Distatrix sancus* wasps caged with caterpillars of *G. alexis* survived for 2 days, but died within 24 h without this food resource.

We also tested *Cotesia cupreus* (Lyle 1925), a parasitoid reared from field-collected caterpillars of *G. alexis*. *Cotesia cupreus* wasps repeatedly stung mature *G. alexis* larvae, but never visited their DNO's in our trials. Again, defensive movements and TO eversions of the lycaenids failed to repel the parasitic wasps. When *C. cupreus* females were caged in petri dishes with mature caterpillars of *P. bellargus*, the wasps often stung these larvae, which again responded with defensive movements and TO eversions. For the most part, *C. cupreus* did not visit the DNO, but on one occasion two wasps antennated the DNO of mature larvae of *P. bellargus* and repeatedly harvested its secretions. *Mesochorus discitergus* (Say) (Ichneumonidae: Mesochorinae), a hyperparasitoid reared from *Distatrix sancus* (via *Callophrys rubi* caterpillars), never showed any interest in caterpillars of *G. alexis* or *P. bellargus*.

Although our observations were made under artificial conditions in the laboratory, they suggest that several parasitoids of the braconid subfamily Microgasterinae (*Apanteles* sp., *Distatrix sancus, Cotesia cupreus*) are able to use their lycaenid hosts in two ways: as a substrate for larval development and as a nutritive resource for the adult wasps. Given the amazing persistence of some wasp-caterpillar associations, feeding of DNO secretions probably occurs under natural conditions as well in female braconid parasitoids of myrmecophilous lycaenid larvae. The limited interest of male wasps in the caterpillar is not surprising given that males rarely, if ever, encounter host larvae in nature.

Several species of the braconid genus *Apanteles* are known parasitoids of myrmecophilous as well as myrmecoxenous lycaenid larvae (Dempster 1971; Pierce & Easteal 1986; Pierce et al. 1987). Unfortunately, little is known about the host-specificity and behavior of most of these lycaenid parasitoids. The *Apanteles* species reared from larvae of *P. coridon* was highly attracted to *P. daphnis* caterpillars as well as to a *P. bellargus* pupa. *Distatrix sancus* and *C. cupreus* wasps even visited the DNO of lycaenid larvae that belonged to different genera than their original hosts. Hence, the Microgasterinae species tested probably are not all species-specific parasitoids. That no *Apanteles* wasps emerged from the *P. daphnis* and *P. bellargus* caterpillars stung during our observations can be attributed to the inadequate larval instar employed in our trials, i.e, *Apanteles* wasps usually parasitize younger instars.

Certain female braconids effectively mimic the tactile signals that ants normally use to elicit the DNO secretions of lycaenid caterpillars. Interestingly, the braconids resemble the ant species *Plagiolepis pygmaea* in size and overall activity pattern, and this ant species often tends caterpillars of *Polyommatus coridon* or *P. hispanus*. Because larvae of European *Polyommatus* and *Glaucopsyche* species are associated unspecifically and facultatively with a number of trophobiotic ant species from at least 8 genera in 3 subfamilies (Fiedler 1991), the signals required to release DNO secretions of such caterpillars cannot be highly specific. Indeed, secretion acts occasionally can be released artificially using vibrating hairs (Schurian unpubl.). However, because the ichneumonid wasps tested so far were unable to mimic the appropriate tactile signals, secretion-feeding of certain Microgasterinae wasps appears to be a peculiarity of these latter parasitoids.

Caterpillars of *Polyommatus coridon, P. hispanus, Glaucopsyche alexis* (all myrmecophiles) and *Callophrys rubi* (a myrmecoxenous species) suffer from parasitism by braconids, ichneumonids, and other parasitoids (this study; Schurian & Fiedler unpubl.), suggesting that the protective effect of tending ants is not perfect. In two other ant-caterpillar mutualisms, attendant ants have been found to be ineffective against parasitoids (Pierce et al. 1987; Nash 1989; DeVries 1991). Possibly, many parasitoids of myrmecophilous caterpillars are adapted to escape ant-attacks. In addition, the species of attendant ants, or the actual population densities of ants, trophobionts, and enemies, may influence the protective benefits of myrmecophily, as has been demonstrated for ant-homopteran interactions (e.g., Bristow 1984; Cushman & Whitham 1989). The attendant ants observed at the *P. coridon* larvae in southern France (*Plagiolepis pygmaea*) are among the smallest ant species of Europe and might thus yield particularly limited protection.

In the Australian lycaenid *Jalmenus evagoras* (Donovan 1805), braconids of the genus *Apanteles* have specialized on using the caterpillars' ant guard as an olfactory oviposition cue (Nash 1989). The larvae and pupae of all lycaenids involved in our experiments (*Polyommatus* spp., *Glaucopsyche alexis, Callophrys rubi*), as well as those of *J. evagoras*, produce substrate-borne vibratory signals. Such caterpillar "calls" might serve as additional cues in the host-locating behavior of some parasitoids. However, because all larvae of *G. alexis* or *C. rubi* that later yielded braconid parasitoids were collected as second instars, caterpillar vibrations appear to play no role as cue for parasitoids in the species tested here. The vibratory abilities of these lycaenid larvae commence with the third instar (Fiedler unpubl.).

Caterpillars of *C. rubi* and *G. alexis* retained their ability to produce vibratory signals even after the braconid larvae had emerged for pupation. In contrast, larvae of *C. rubi* parasitized by the tachinid fly *Aplomyia confinis* (Fallén 1820) lost their ability to vibrate a few days before the parasitoids left (Fiedler unpubl.). Clearly, the ecological and behavioral relationships between lycaenids, ants, and parasitoids merit further attention of lepidopterists, as evidenced by the discovery of the novel type of caterpillar-braconid interaction described here.

We thank E. Haeselbarth, K. Horstmann, J. Papp, H.-P. Tschorsnig, and H. Schnee for taxonomic and biological information on the braconids, ichneumonids, and tachinids involved. Special thanks go to J. Klein who kindly drew the figure, and to P. J. DeVries for his helpful comments on the manuscript. K. F. gratefully acknowledges support from the Leibniz Award of the DFG to B. Hölldobler.

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Received for publication 20 May 1992; revised and accepted 24 October 1992.

Journal of the Lepidopterists' Society
47(2), 1993, 154–160

EVIDENCE FOR USE OF WATER BALLAST BY MONARCH BUTTERFLIES, *DANAUS PLEXIPPUS* (NYMPHALIDAE)

Additional key words: migration, flight stability, gliding, soaring, lipids.

To ensure stable flight characteristics, the Center of Gravity (CG) of an aircraft must be located relatively close to the center of lift (Falk & Matteson 1971; Anonymous 1991). This constraint also applies to gliding butterflies. Gibo and Pallett (1979) investigated