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Myrmecophilous behaviours in caterpillars of the butterfly, *Polyommatus icarus* (Rottemburg, 1775) : temporal patterns and age dependency (Lepidoptera : Lycaenidae)

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Summary

Mutualistic interactions between *Lasius flavus* (Fabricius, 1782) ants and caterpillars of the Common Blue *Polyommatus icarus* (Rottemburg, 1775), a facultatively myrmecophilous lycaenid butterfly, were studied in a standardised laboratory assay. Caterpillars delivered more and larger nutritious secretion droplets from their dorsal nectar organ in the initial phase of an interaction than later on. Activity of their tentacle organs followed the same pattern. Myrmecophilous behaviours remained almost constant throughout the feeding phase of the final larval instar, whereas non-feeding prepupal larvae showed a more than fivefold increase in secretion rates from the nectar organ and a three- to fivefold increase in the frequency of tentacle eversions. The frequency of secretion acts as well as tentacle eversions was drastically reduced when larvae were raised on low-quality food. The results indicate that *P. icarus* caterpillars highly flexibly respond with their mutualistic behaviours according to actual needs and resource availability. The close relation between the activity of the nectar organ and the tentacles is further evidence that the latter organs produce “honest signals” which advertise the capacity of a caterpillar to provide food rewards to attendant ants.

Zusammenfassung

Mutualistische Interaktionen zwischen der Ameisenart *Lasius flavus* (Fabricius, 1782) und Raupen des fakultativ myrmekophilen Bläulings *Polyommatus icarus* (Rottemburg, 1775) wurden in standardisierten Laborversuchen quantitativ analysiert. In den ersten Minuten einer Assoziation sezernierten die Raupen häufiger und größere Sekrettropfen aus ihrem Nektarorgan als in fest etablierten Vergesellschaftungen. Die Aktivität der Tentakelorgane nahm parallel zur Sekretabgaberate ab. Die Zeitmuster der myrmekophilen Verhaltensweisen der Raupen blieben während der fraßaktiven Phase des vierten Larvalstadiums trotz einer Vervierfachung ihres Gewichts nahezu konstant, während in der präpupalen Phase die Sekretabgaberate und die Aktivität der Tentakelorgane für wenige Stunden auf das Drei- bis Fünffache gesteigert wurden. Unter Nahrungsstreß (Aufzucht auf maturen Blättern von *Medicago sativa*) war die Sekretabgaberate wie auch die Aktivität der Tentakelorgane drastisch reduziert, folgte aber denselben Zeit- und Entwicklungsmustern wie bei Raupen auf optimaler Diät (Blütenstände von *M. sativa*). Diese Befunde zeigen, daß die Raupen von *P. icarus* ihre myrmekophilen Verhaltensweisen plastisch an ihre momentane Situation (Stabilität der Ameisenassoziation, Entwicklungsstand, Ressourcenverfügbarkeit) anpassen. Aus der engen Korrelation zwischen der Aktivität der Tentakelorgane und der Sekretabgaberate aus dem Nektarorgan läßt sich ferner ableiten, daß die Tentakel bei dieser Art als „verläßliche Signale" wirken könnten, die den Ameisenpartnern die Rentabilität einer Raupe als Futterquelle anzeigen.

Introduction

Ants maintain a variety of mutualistic interspecific interactions with organisms such as plants (Beattie, 1985 ; Huxley & Cutler, 1991), sap-sucking homopterans or butterfly caterpillars (Hölldobler & Wilson, 1990). An integral part of these mutualisms is the trade of two commodities : ants receive nutritious substances and, in turn, protect their mutualists against enemies like herbivores, predators, or parasitoids (e.g. Pierce *et al.*, 1987).

Interactions with ants, termed myrmecophily, are widespread in two butterfly families, viz. Lycaenidae and Riodinidae (for reviews see Pierce, 1987 ; Fiedler, 1991). In butterflies, myrmecophily mostly occurs during the later larval instars and is mediated by specialized exocrine glands, the so-called myrmecophilous organs. Among the better studied family Lycaenidae, interactions with ants range from mutualistic to commensalic or parasitic. More than 50% of the 4.500 described lycaenid species are myrmecophilous, and in the great majority these interactions with ants are believed to be mutualistic (Fiedler, 1991). While the caterpillars of certain butterfly species always associate with specific host ants and can hardly survive without these (so-called obligate myrmecophiles), larval ant-associations are facultative and unspecific in most lycaenid species. This means that caterpillars can survive without ants, that at any given time varying fractions of a caterpillar population are not tended by ants, and that the caterpillars are unspecifically tended by a variety of ant genera as represented in the local ant fauna (Fiedler, 1991).

Several types of glandular epidermal organs (Malicky, 1969 ; Cottrell, 1984 ; Ballmer & Pratt, 1992) mediate, in combination with substrate-borne vibratory signals (DeVries, 1990), the associations between lycaenid caterpillars and ants (Fiedler *et al.*, 1996). Among these glands, the dorsal nectar organ (DNO hereafter) on the seventh abdominal segment is the major source of energetic reward in the caterpillar-ant interactions (Fiedler & Maschwitz, 1988a). From their DNO the caterpillars secrete discrete droplets of a secretion rich in carbohydrates and/or amino acids (Maschwitz *et al.*, 1975 ; Cushman *et al.*, 1994). In addition, caterpillars of many lycaenid species possess a pair of eversible tentacle organs (TOs) located on the eighth abdominal segment. The TOs are most often everted when caterpillars are disturbed or while moving, and attendant ants respond with increased activity and attention (Henning, 1983 ; Fiedler & Maschwitz, 1988b). The activity of the DNO and the TOs can easily be quantified.

In contrast to the excretions ("honeydew") which mediate the ant-homopteran mutualisms, secretions from the myrmecophilous organs of butterfly caterpillars are produced at some metabolic costs. Therefore, caterpillars should be under selection to minimise their energetic investment into the symbiosis according to actual needs. In concordance with this expectation, Leimar & Axén (1993) found that caterpillars of *Polyommatus icarus* adjust their DNO secretion and TO eversion rates to their actual tending level and also respond to simulated attacks with increased secretory activity. Further examples for such plasticity of myrmecophilous behaviours in response to larval tending levels have been documented from other species (Fiedler & Hagemann, 1995, Fiedler & Hummel, 1995). This "strategic behaviour" may help to minimise costs. In fact, caterpillars of *P. icarus* do not suffer from developmental net costs when tended by ants, but even overcompensate for their investment into myrmecophily : ant-tended males grow larger than untended controls (Fiedler & Hölldobler, 1992). Similar observations have in the meantime been made on a variety of lycaenid species (Wagner, 1993 ; Cushman *et al.*, 1994 ; Fiedler & Saam, 1994), indicating that mechanisms to minimise costs are widespread.

We here address two questions related to this topic. Firstly, how do secretion rates change with time when a caterpillar-ant association becomes established ? Secondly, how do secretion rates change with larval growth ? We studied both these questions under two different feeding regimes, viz. high quality food conditions and severe food stress.

Material and methods

Study organisms

Butterflies. The Palearctic Common Blue butterfly, *Polyommatus icarus* (Rottemburg, 1775) is widespread in Eurasia and can be bred for a number of generations per year in the laboratory. In nature, caterpillars of *P. icarus* are facultatively tended by ants belonging to at least 4 genera (*Lasius, Formica, Plagiolepis, Myrmica*) in 2 subfamilies (Fiedler, 1991), and some caterpillars are typically found untended (Emmet & Heath, 1989). Various aspects of ant-interactions of *P. icarus* have been studied intensively in the laboratory (Fiedler, 1990, 1991 ; Fiedler & Hölldobler, 1992 ; Leimar & Axén, 1993 ; Fiedler & Saam, 1994 ; Burghardt & Fiedler, 1996). Our culture originated from mated females caught in the field (origin : northern Bavaria). Females were allowed to oviposit in glass cages kept in a greenhouse (for principals of the rearing procedure see Schurian, 1989). Eggs were collected daily and transferred to a climatic chamber (15 :9 h L :D cycle, 22.5°C constant). After hatching, groups of caterpillars were transferred into translucent closed plastic vials (125 ml) lined with moist filter paper, where they were raised on ad libitum food until they had moulted into the third instar. With the beginning of the third instar, the DNO and TOs become functional in *P. icarus*, and from that time onwards all experimental caterpillars were kept individually until pupation. The caterpillars had contact to ants only during the daily experimental period (15 min per individual).

Caterpillars of *P. icarus* usually feed on inflorescences or young foliage of various herbaceous Fabaceae species (Martín, 1984 ; Ebert & Rennwald, 1991), and food quality is known to strongly affect myrmecophily in that species (Fiedler, 1990 ; Burghardt & Fiedler, 1996). We here report on caterpillars under two feeding regimes (see Burghardt & Fiedler, 1996 for experiments with further plant species). One group ($n = 20$) was raised on *Medicago sativa* L. inflorescences throughout the whole larval development. This is a natural high-quality food for the fast growing caterpillars. A second group ($n = 19$) was fed mature foliage of the same plant species (*M. sativa*) sampled in the same habitat. As expected, this food treatment caused substantial stress to the larvae, which pupated at much lower weights (mean ± S.E. = 59.2 ± 1.8 mg, $n = 19$) than on the high-quality diet (91.1 ± 3.2 mg, $n = 20$; U-test, $p < 0.002$).

Ants. For our experiments we chose *Lasius flavus* (Fabricius, 1782). This widespread ant species of open grasslands feeds mainly on honeydew excreted by specific root aphids, the diet being supplemented by surplus aphids to obtain nitrogen (Pontin, 1958, 1978 ; Godske, 1992). Because of their highly developed trophobiotic behaviour, *L. flavus* ants readily associate with lycaenid caterpillars in laboratory assays. It must be noted, however, that due to their mostly subterranean life-habits *L. flavus* ants rarely tend lycaenid caterpillars in nature (Fiedler, 1991).

We kept the ants in their natural earth nests as taken from the field (origin : northern Bavaria). They were maintained in large plastic arenas (66 cm × 44 cm 12 cm) with a bottom layer (1-2 cm) of plaster of Paris. Ants received honey water and dead cut cockroaches as needed. We used three different ant colonies for our experiments.

Experimental procedure. For experiments, we carefully transferred 15 foraging worker ants with a fine brush from the nest arena to a small test arena (a plastic box of 10 cm × 10 cm × 6 cm with a moist bottom layer of plaster of Paris). Arena walls were coated with Fluon to prevent the ants from escape. The ants were left undisturbed for 5-10 min. After that time all signs of alarm behaviour had faded off and the ants walked around in the arena in search for food. Then, a single caterpillar was placed in the centre of the arena.

Starting with the first contact between the caterpillar and an ant, we determined the number and timing of DNO secretion droplets as well as of TO eversions (eversion of one or both tentacle organs were equally counted as one event) during a test period of 15 min. After each experiment, we noted the age of the caterpillar (in days since hatching from the egg) and its weight (determined to the nearest 0.1 mg on an electronic Sartorius Basic 61 balance). We also measured the diameter (to the nearest 0.05 mm) of as many DNO secretion droplets as possible, using a calibrated eyepiece.

Observations were made under a stereomicroscope at 10fold magnification under natural daylight and at room temperatures (22-25°C). The caterpillars had no access to food during the 15 min trials, but were returned to their rearing vials with ad libitum food immediately after the end of each experiment. Each set of ants was used for a maximum of three subsequent experiments, resulting in a maximum time of 60 min being separated from their home colony. There was no indication that the behaviour of ants towards the larvae changed during this period. Then, the ants were returned to their colony and replaced by a new set of 15 foragers.

To study the development of myrmecophilous behaviours throughout the fourth (= final) larval instar, we tested the larvae at various times between the moult into the fourth instar and pupation. Because the larvae did not grow equally fast, we could not use absolute larval age as a reliable basis for comparisons. Under our rearing conditions, most larvae passed the final instar in 5.5 d on *Medicago sativa* inflorescences, and in 6.5 d when feeding on *M. sativa* foliage, but on both diets some larvae took 1-2 d longer. Therefore, we divided the final instar larvae in 4 relative size classes according to their weight at the time of testing divided by their final prepupal weight. This gives a dimensionless quotient of "relative weight". We scored the larvae in the following classes : class I (relative weight < 0.4 ; corresponding to the first day of the fourth instar) ; class II (relative weight 0.4-0.8 ; corresponding to the following 1-2 days) ; class III (relative weight > 0.8 ; corresponding to the final 1-2 days of the feeding phase of the fourth instar) ; and class IV ("non-feeding prepupae"). "Non-feeding prepupae" denotes larvae which have stopped feeding and which were characterised by the following morphological and behavioural traits : these caterpillars are light-greenish, the whitish color markings of the feeding phase have vanished, the caterpillars' cuticle looks glossy, they have left the foodplant and hide within the filter paper, and they have excreted their last frass pellet, which looks red due to the excretions of the Malpighian tubules. Prepupae were only tested as long as they were still able to crawl and had not yet spun a silk girdle for pupation. Experimental data were analysed using two-tailed non-parametric test procedures (U-tests, H-tests, Spearman's rank correlation : Sachs, 1992), except where stated otherwise.

Results

Temporal patterns of myrmecophilous interactions

We noted no significant differences with regard to DNO secretion rates, TO eversion rates, or actual levels of ant-attendance between larvae of the relative weight classes I-III, i.e. within the feeding phase of the fourth larval instar (see below). Therefore, we present data on the time course of caterpillar-ant interactions only for fully-fed mature larvae (weight class III) and non-feeding prepupae (class IV). Results for the relative size classes I and II are entirely analogous (Burghardt, 1994). In both age classes and on both larval diets we observed similar temporal patterns in the secretory behaviour of *P. icarus* larvae. DNO secretion rates were always much higher in the initial 3 min of an interaction than later on (Fig. 1). In mature larvae (class III) reared on *Medicago sativa* inflorescences, the DNO secretion rates dropped from 3 droplets/3 min to a constant low level of 0.6 droplets/3 min within 3 min. In prepupae the decrease in secretory activity from 7 droplets/3 min was less steep and reached a constant low level of 2.5 droplets/3 min only after 9 min. On *M. sativa* foliage, the temporal pattern was identical, but the secretion rates were overall much lower (Fig. 1).

The frequency of TO eversions followed roughly the same temporal pattern as the DNO secretion rates (Fig. 2). TO eversions occurred more often in the initial 3 min of an interaction than later on. In addition, the proportion of larvae which did not evert their TOs at all increased from 24% (flowers) or 33% (foliage) in the initial 3 min interval to 57% (flowers) or 90% (foliage) later on. As with the DNO secretions, the drop in TO activity was pronounced after 3 min in mature larvae, but less steep and abrupt in non-feeding prepupae (Fig. 2).

In our experimental assay with a surplus of 15 ant mutualists available in a small arena, actual tending levels of caterpillars showed almost no change through experimental time (data not shown ; Burghardt, 1994). Within less than 1 min after the first encounter, each caterpillar was constantly visited by 4-10 ants, and there was neither an increase nor a decrease in the tending levels within the 15 min 1 h experiments.

Age and food effects on secretory behaviour

DNO secretion rates (Fig. 3) were statistically not different between the relative weight classes I-III within the fourth larval instar (Kruskal-Wallis test, $H = 0.223$, $p > 0.8$ for caterpillars reared on flowers ; $H = 0.886$, $p > 0.6$ for larvae on foliage). In the prepupal phase, however, DNO secretion rates increased distinctly in comparison to mature feeding larvae (Mann-Whitney U-test, $Z = 4.564$, $p < 0.0001$ for animals fed flowers ; $Z = 5.321$, $p < 0.0001$ for caterpillars reared on foliage).

Largely the same age pattern occurred with TO eversion rates (Fig. 4). These were practically constant throughout the feeding phase of the fourth instar (Kruskal-Wallis test, $H = 1.179$, $p > 0.5$ for caterpillars reared on flowers ; $H = 0.065$, $p > 0.9$ for caterpillars reared on foliage). In flower-fed caterpillars, there was a highly significant increase in TO eversion rates from mature feeding to prepupal non-feeding larvae (Mann-Whitney U-test, $Z = 3.404$, $p < 0.001$). For foliage-fed caterpillars, this same difference was only observed as a statistical trend

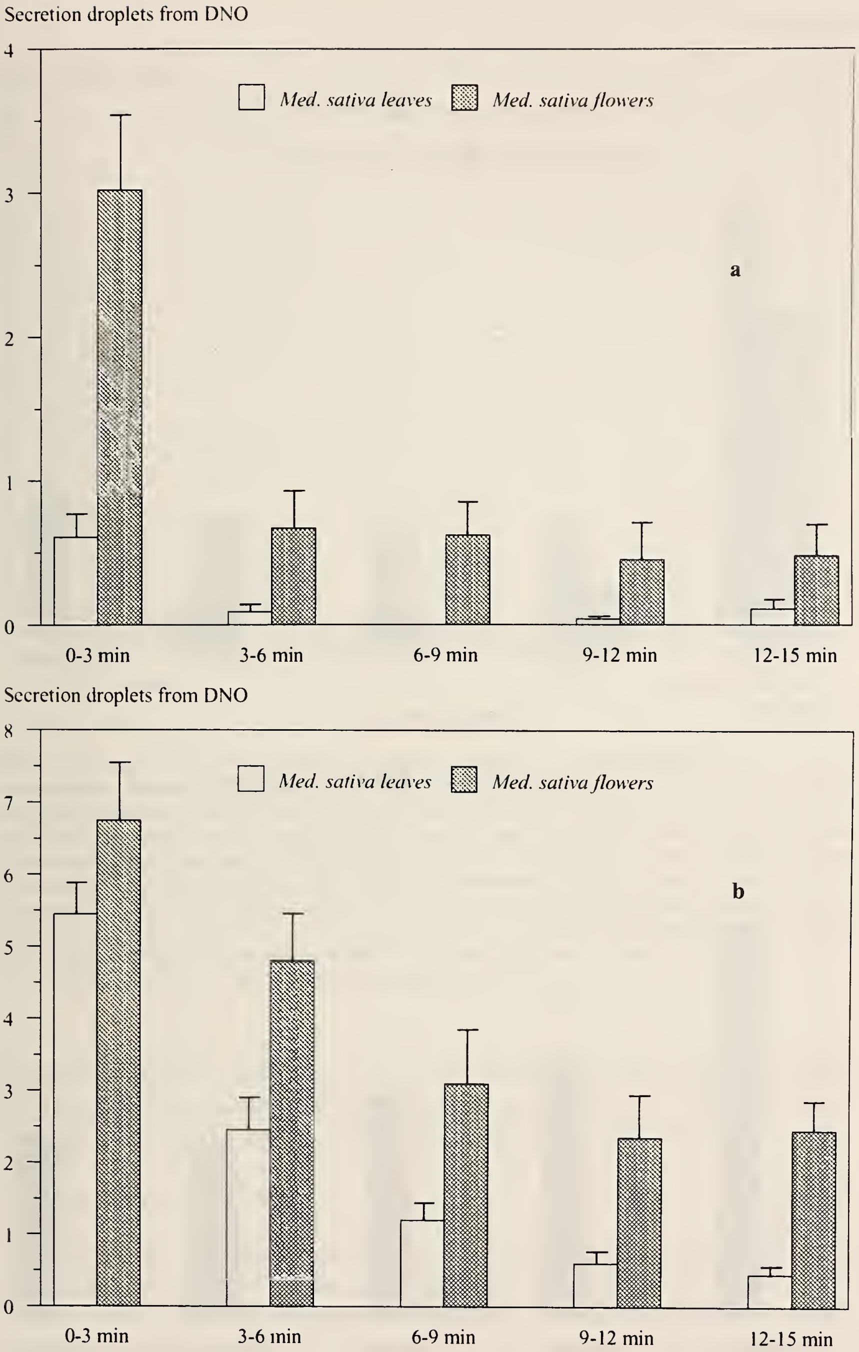

Fig. 1. Temporal pattern of the delivery of secretion droplets from the dorsal nectar organ (DNO) by *Polyommatus icarus* caterpillars (fourth instars) to *Lasius flavus* ants (means + S.E.). Experiments lasted 15 min. White bars : caterpillars raised on *Medicago sativa* foliage (food stress treatment) ; cross-hatched bars : caterpillars fed *M. sativa* flowers (high-quality food treatment). A) mature feeding caterpillars, weight class III, relative weight > 0.8 ($n = 21$ for both food treatments). B) non-feeding prepupal larvae, weight class IV ($n = 20$ for both food treatments).

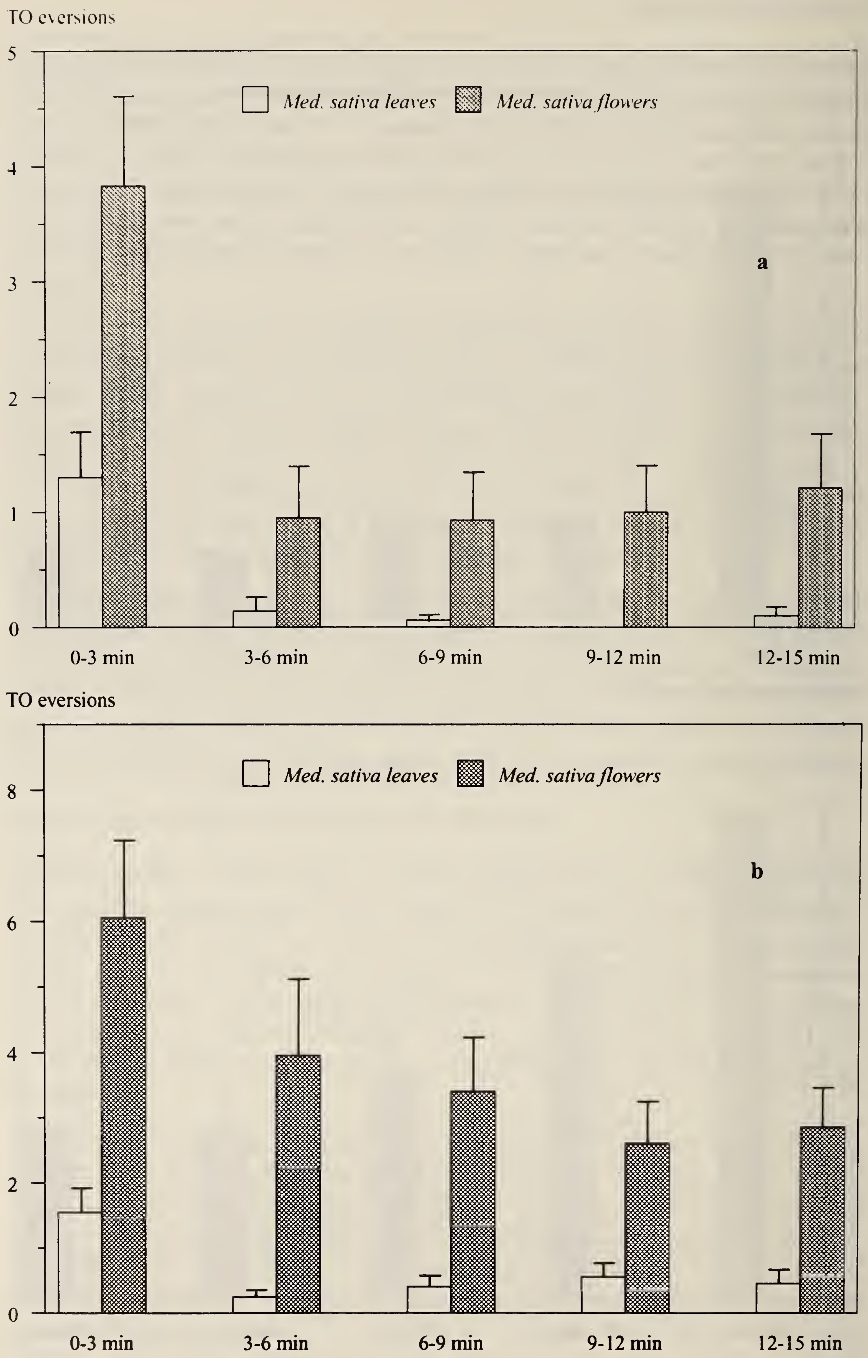

Fig. 2. Temporal pattern of eversions of the tentacle organs (TO) by *P. icarus* caterpillars (means + S.E.). White bars : caterpillars raised on *Medicago sativa* foliage (food stress treatment) ; cross-hatched bars : caterpillars fed *M. sativa* flowers (high-quality food treatment). A) mature feeding caterpillars, weight class III (n = 21 for both food treatments). B) non-feeding prepupal larvae, weight class IV (n = 20 for both food treatments).

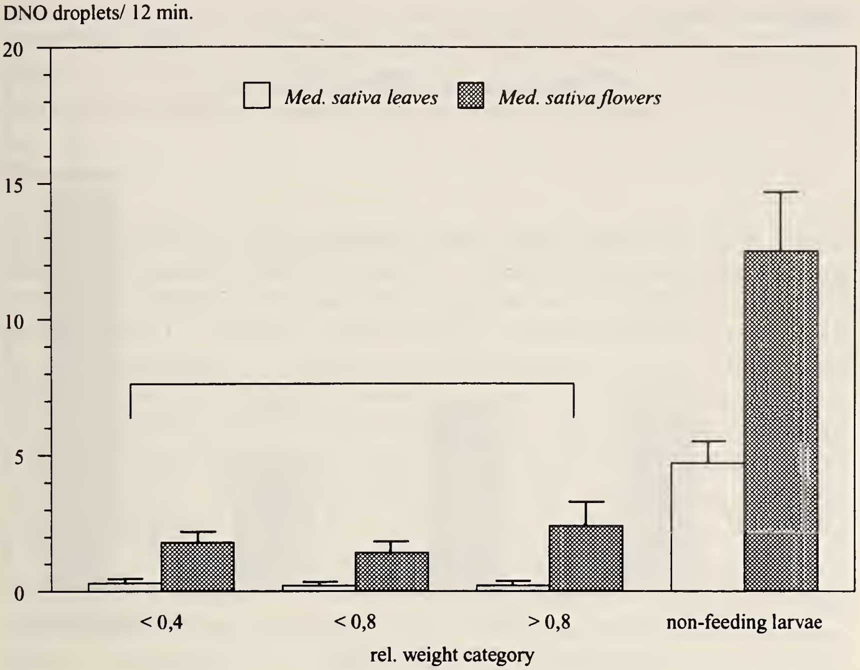

Fig. 3. Number of DNO secretion droplets (means + S.E.) delivered by *P. icarus* caterpillars (fourth instars) of four relative weight classes during the final 12 min of experimental interactions with *L. flavus* ants. Relative weights are calculated as the quotient of actual larval weight at the end of an experiment, divided by the final prepupal weight reached by the same individual. The square bracket above the first three categories denotes that, on each food treatment, no significant differences were observed between the weight classes. Empty bars : caterpillars were fed *M. sativa* leaves (class I : n = 22, class II : n = 22, class III : n = 21, non-feeding prepupae : n = 20). Cross-hatched bars : caterpillars raised on *M. sativa* flowers (class I : n = 12 ; class II : n = 20 ; class III : n = 21 ; non-feeding larvae : n = 20).

(Mann-Whitney U-test, $Z = 1.434$, one-tailed $p < 0.08$) due to the high variance. Nevertheless, overall TO eversion rates were on average twice as high in prepupal larvae in this latter series of experiments.

Size of secretion droplets

We measured the diameter of 107 DNO secretion droplets delivered by fourth instars of *P. icarus*. 74 of these occurred in the initial 3 min of an experiment, 33 droplets in the final 12 min. Droplet diameters (Ø) did not differ significantly among larvae in the relative weight classes II-IV (mean diameters ± S.E. ; class II : Ø = 0.239 ± 0.019 mm,

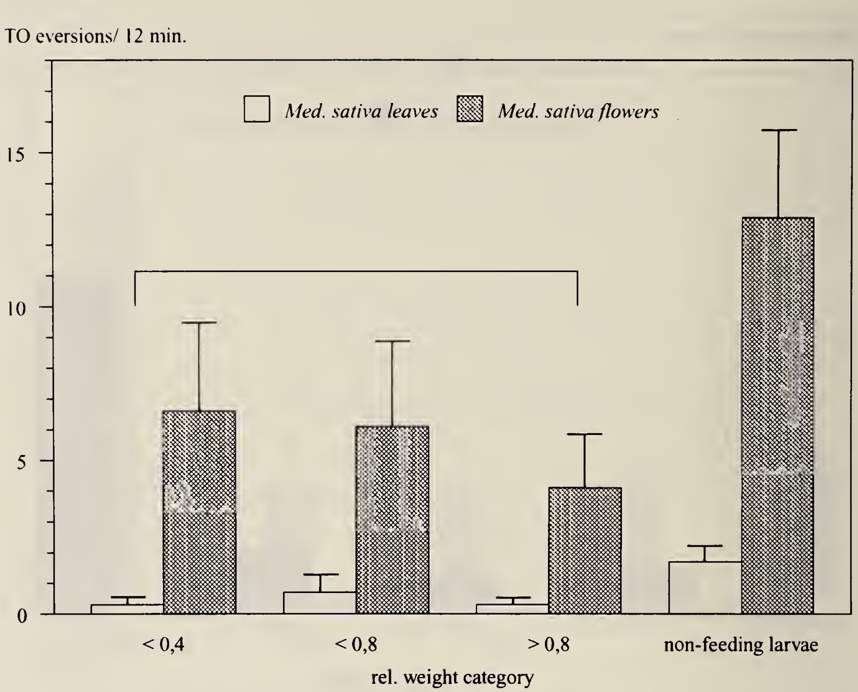

Fig. 4. Number of tentacle (TO) eversions (means + S.E.) of *P. icarus* caterpillars of four relative weight classes during the final 12 min of experimental interactions with *L. flavus* ants. The square bracket above the first three categories denotes that, on each food treatment, no significant differences were observed between the weight classes. Empty bars : caterpillars were fed *M. sativa* leaves (class I : n = 22, class II : n = 22, class III : n = 21, non-feeding prepupae : n = 20). Cross-hatched bars : caterpillars raised on *M. sativa* flowers (class I : n = 12 ; class II : n = 20 ; class III : n = 21 ; non-feeding larvae : n = 20).

n = 14 ; class III : Ø = 0.258 ± 0.012 mm, n = 13 ; class IV : Ø = 0.252 ± 0.011, n = 32 ; Kruskal-Wallis test, H = 0.986, $p > 0.6$). The combined average droplet diameter for these three weight classes was hence Ø = 0.250 ± 0.004 mm during the initial 3 min of an interaction, corresponding to a spherical volume of 0.0082 µl. Young fourth instars (class I), however, produced significantly smaller droplets (Ø = 0.170 mm ± 0.008 mm, n = 15 ; Mann-Whitney test, $U_{14;15} = 42$, $p < 0.01$), with a spherical volume of 0.0026 µl.

DNO secretion droplets delivered during the final 12 min of an experiment were significantly smaller (Ø = 0.197 ± 0.008 mm, volume 0.004 µl, n = 33 [4 in class III, 29 in class IV]) than those in the initial phase of an interaction (Mann-Whitney U-test, Z = 3.899, $p < 0.001$). These measurements show that larvae of *P. icarus* not only reduce the number of DNO secretion droplets, but also droplet size as soon as a stable ant-association has been established.

Discussion

Our experimental results revealed three major patterns : 1) *P. icarus* caterpillars quickly reduce the activity of their myrmecophilous organs as soon as a stable association with ants has been established ; 2) DNO secretion and TO eversion rates distinctly increase when the larvae reach the prepupal stage, yet remain constant throughout the feeding phase despite a more than fourfold increase in body mass of *P. icarus* caterpillars ; 3) these temporal and developmental patterns remain unaffected if the larvae are reared under food stress, but the amount of secretions to be allocated into the mutualism critically depends on the food resources available to the larvae (see also Fiedler, 1990 ; Burghardt & Fiedler, 1996).

The temporal patterns of secretory behaviours are concordant with the findings of Leimar & Axén (1993) that myrmecophilous caterpillars behave "strategically" viz. adjust their DNO secretion and TO eversion rates to their actual needs. In our experimental conditions, stable caterpillar-ant associations established within 1-3 min after the initial contact, and after this period there was no need for the caterpillars to continue with their potentially costly behaviours at the same high rate. Flower-fed mature *P. icarus* larvae spent on average 0.024 µl DNO secretions in the initial 3 min compared to 0.009 µl in the subsequent 12 min. For prepupal caterpillars, the respective estimates are 0.058 µl in the initial 3 min and 0.050 µl in the subsequent 12 min. This temporal pattern did not depend on the absolute amount of resources available to the caterpillars, since we observed it with larvae at all sizes and ages, and on the high-quality food (inflorescences) as well as under severe nutritional stress (foliage). Furthermore, the same pattern occurs in a number of lycaenid butterfly species tested so far under the same experimental conditions (*Celastrina argiolus* : Burghardt, 1994 ; *Zizeeria knysna* : Fiedler & Hagemann, 1995 ; *Aricia agestis* : Fiedler & Hummel, 1995 ; *Polyommatus coridon, P. cornelia* : Fiedler unpubl.). Therefore, this strategy appears to be widespread among butterfly species whose larvae live in facultative symbioses with ants.

DeVries (1988) described the establishment of interactions between ants and myrmecophilous caterpillars in the family Riodinidae as an "enticement and binding" process, where the rewarding with nutritious secretions is particularly important during the initial phase of an association, whereas later on a combined action of all myrmecophilous organs ascertains the integrity of the association in time. Our observations on a range of lycaenid species, including *P. icarus*, indicate that a similar strategy is prevalent in the butterfly family Lycaenidae as well. To offer more and larger droplets after the first contacts between a caterpillar and an ant will strengthen the binding between the two partners and may even induce the scout ant to recruit additional nestmates (Fiedler & Maschwitz, 1988a), whereas later on the secretion rate and droplet size are modulated to lower levels.

Even at the very beginning of an interaction with ants, the caterpillars probably do not deliver the complete stored secretion from their DNO reservoirs. This conclusion can be drawn when the secretion volumes observed in our experiments are compared with morphometric data on the size of the DNO in *P. icarus*. According to Malicky (1969 : 253), each glandular bladder of the DNO is 0.72 mm long and 0.2 mm wide. The DNO has four such ellipsoidic bladders with a cumulative reservoir volume of approximately 0.06 µl. The secretion volumes of the initial 3 min in our experiments were distinctly lower than this reservoir volume for feeding mature caterpillars (0.024 µl), but almost as large (0.058 µl) in prepupae.

Could the enhanced initial secretion rates be a response to unavoidable handling at the beginning of an experiment rather than an adaptive trait ? Although handling disturbance might have contributed to the pattern observed (cf. Leimar & Axén, 1993), the above calculation indicates that at least during the feeding phase larvae retain some "currency" for future use. Furthermore, while being constantly attended by ants, only 50% of *P. icarus* larvae responded to tactile disturbance with one surplus secretion droplet (Fiedler, unpubl. data), whereas all larvae in the experiments detailed above produced on average two additional droplets in the initial 3 min interval. Hence, even if response to handling did affect our observations, it seems unlikely that this would be the sole source of the effect (see detailed discussion in Fiedler & Hagemann, 1995).

Non-feeding prepupal larvae enhance their activity relevant to the mutualism for a short period of time (less than 12 h in *P. icarus*). Again, this finding is paralleled by results obtained with *Zizeeria knysna* (Fiedler & Hagemann, 1995), *Polyommatus cornelia* (Fiedler *et al.*, 1994), *P. coridon* (Fiedler, unpubl.), and *Aricia agestis* (Fiedler & Hummel, 1995). Inferring from the significant weight losses of ant-tended prepupae, a substantial investment into myrmecophily does probably also occur at this developmental stage in the Nearctic *Hemiargus isola* (Wagner, 1993).

What adaptive significance, if any, may this enhanced prepupal investment into myrmecophily have? We suggest that enhanced prepupal secretory activity assists to strengthen the caterpillar-ant association during the most vulnerable time of the life-cycle. Firstly, prepupae and pupae are immobile and thus unable to escape from, or defend against, attacks by predators or parasitoids. Secondly, freshly moulted pupae have an extraordinarily soft cuticle which renders them highly susceptible to fatal damage. Thirdly, all species where enhanced secretory activity in the prepupal phase has so far been observed, descend from their hostplants for pupation, which then takes place on the soil, in the litter or at the hostplant base. In such locations, the likelihood of encounters with ground-foraging ants is much higher than on the hostplants. Therefore, the prepupae require effective mechanisms to appease ants and may gain further selective advantages if ant-associations can be maintained and extended far into the pupal stage (see Fiedler, 1988, and Wagner, 1995, for myrmecophily of lycaenid pupae or prepupal larvae).

In view of earlier experiments on the influence of food quality on the ability of *P. icarus* caterpillars to produce DNO secretions (Fiedler, 1990), it was not surprising that foliage-fed caterpillars were much inferior to flower-fed siblings with respect to their myrmecophilous properties (see also Burghardt & Fiedler, 1996). Strong effects of hostplant quality on the attractiveness of lycaenid caterpillars to ants have also been reported for the Australian obligate myrmecophile *Jalmenus evagoras* (Baylis & Pierce, 1991, 1993). The important notion from our experiments is that the temporal as well as developmental patterns of myrmecophilous behaviours remain unchanged under food stress, although the absolute and relative investment into myrmecophily are very sensitive to the nutritional environment of the larvae.

Because we did not quantify the nutrient content in the minute secretion droplets, we cannot rule out that changes in droplet size or secretion rate might be accompanied by changes in nutrient concentrations. This also applies to the experiments of Leimar & Axén (1993). The possible variance of food rewards should evidently be studied in the future. However, in our experiments we never observed that the ants would reject a single secretion droplet. Hence, even if variation in secretion quality should have occurred, all droplets were highly attractive to the attendant ants.

In our experiments, TO eversion rates almost exactly followed the frequency patterns of DNO secretions : an enhanced initial TO activity was followed by a rapid decrease, more frequent TO eversions occurred in the prepupal phase, and TO activity was much reduced under food stress. Although the exact function of the TOs in lycaenid-ant mutualisms is still under discussion, there is ample evidence that these organs alert and attract tending ants (Fiedler & Maschwitz, 1988b ; Ballmer & Pratt, 1992). Fiedler (1991 : 8-29) observed that TO eversion rates in *P. icarus* larvae were significantly correlated with the stability of their ant-associations. Since the action of the TOs involves the production of some communicative signal as well as muscular activity, these organs pose an additional energetic cost on the caterpillars, even if this cost is likely to be small. It should therefore be adaptive that caterpillars restrict TO activity to times when these organs may assist in establishing or stabilising caterpillar-ant associations, like at the beginning of an interaction or when entering the prepupal phase. Under severe food stress, caterpillars should restrict the use of their TOs to spare energy, and this is exactly what we observed in our rearings on *Medicago sativa* foliage. In addition, Leimar & Axén (1993) demonstrated that TO activity is enhanced when caterpillars are subject to a simulated attack. Finally, we observed significant correlations between DNO secretion and TO eversion rates across the tested individuals (flower-fed prepupae : $r_S = 0.594$, $n = 20$, $p < 0.01$; foliage-fed prepupae : $r_S = 0.584$, $n = 20$, $p < 0.05$). Collectively, these observations suggest that TO eversions may advertise, as an honest signal, the profitability of a *P. icarus* larva as nectar resource to its attendant ants.

Our experimental data reveal that myrmecophilous behaviours of *P. icarus* caterpillars show characteristic temporal and developmental plasticity, that the patterns emerging from this plasticity are presumably adaptive, and that the resources available to the larvae are an important constraint on the amount of investment, but do not change the patterns of its delivery. These observations are in accordance with previous work on strategic behaviour in that mutualism (Leimar & Axén 1993) and on developmental costs of myrmecophily (Fiedler & Hölldobler, 1992 ; Fiedler & Saam, 1994). Furthermore, the occurrence of similar effects in a number of additional facultatively myrmecophilous Lycaenidae species also suggest that the patterns observed in *P. icarus* are of general significance for unspecific mutualistic caterpillar-ant interactions, which are by far the most widespread type of interactions with ants in the butterfly family Lycaenidae (Fiedler, 1991).

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References

Ballmer, G. R. & Pratt, G. F., 1992. Quantification of ant attendance (myrmecophily) of lycaenid larvae. *J. Res. Lepid.* 30 : 95-112.

Baylis, M. & Pierce, N. E., 1991. The effect of host-plant quality on the survival of larvae and oviposition by adults of an ant-tended lycaenid butterfly, *Jalmenus evagoras. Ecol. Entomol.* 16 : 1-9.

Baylis, M. & Pierce, N. E., 1993. The effects of ant mutualism on the foraging and diet of lycaenid caterpillars. In Stamp, N. E. & Casey, T. M. (Eds.) : Caterpillars, pp. 404-421. Chapman and Hall, New York/London.

Beattie, A. J., 1985. The evolutionary ecology of ant-plant mutualisms. Cambridge University Press, Cambridge.

Burghardt, F., 1994. Untersuchungen zum Einfluß der Futterqualität auf die Myrmekophilie von Bläulingsraupen. Diplom thesis, University of Würzburg.

Burghardt, F. & Fiedler, K., 1996. The influence of diet on growth and secretion behaviour of myrmecophilous *Polyommatus icarus* caterpillars (Lepidoptera : Lycaenidae). *Ecol. Entomol.* 21 : 1-8.

Cottrell, C. B., 1984. Aphytophagy in butterflies : its relationship to myrmecophily. *Zool. J. Linn. Soc.* 79 : 1-57.

Cushman, J. H., Rashbrook, V. K. & Beattie, A. J., 1994. Assessing benefits to both participants in a lycaenid-ant association. *Ecology* 75 : 1031-1041.

DeVries, P. J., 1988. The larval ant-organs of *Thisbe irenea* (Lepidoptera : Riodinidae) and their effects upon attending ants. *Zool. J. Linn. Soc.* 94 : 379-393.

DeVries, P. J., 1990. Enhancement of symbioses between butterfly caterpillars and ants by vibrational communication. *Science* 248 : 1104-1106.

Ebert, G. & Rennwald, E. (Eds.), 1991. Die Schmetterlinge Baden-Württembergs, Band 2 : Tagfalter II. E. Ulmer, Stuttgart.

Emmet, A. M. & Heath, J. (Eds.), 1989. The moths and butterflies of Great Britain and Ireland. Vol. 7/1 : The butterflies. Harley Books, Colchester.

Fiedler, K., 1988. Die Beziehungen von Bläulingspuppen (Lepidoptera : Lycaenidae) zu Ameisen (Hymenoptera : Formicidae). *Nachr. entomol. Ver. Apollo Frankfurt* N. F. 9 : 33-58.

Fiedler, K., 1990. Effects of larval diet on the myrmecophilous qualities of *Polyommatus icarus* caterpillars (Lepidoptera : Lycaenidae). *Oecologia* 83 : 284-287.

Fiedler, K., 1991. Systematic, evolutionary, and ecological implications of myrmecophily within the Lycaenidae (Insecta : Lepidoptera : Papilionoidea). *Bonner zool. Monogr.* 31 : 1-210.

Fiedler, K. & Hagemann, D., 1995. The influence of larval age and ant number on myrmecophilous interactions of the African Grass Blue butterfly, *Zizeeria knysna* (Lepidoptera : Lycaenidae). *J. Res. Lepid.* 31 : 213-232.

Fiedler, K. & Holldobler, B., 1992. Ants and *Polyommatus icarus* immatures (Lycaenidae) – sex-related developmental benefits and costs of ant-attendance. *Oecologia* 91 : 468-473.

Fiedler, K., Holldobler, B. & Seufert, P., 1996. Butterflies and ants : the communicative domain. *Experientia* 52 : 14-24.

Fiedler, K. & Hummel, V., 1995. Myrmecophily in the brown argus butterfly, *Aricia agestis* (Lepidoptera : Lycaenidae) : effects of larval age, ant number and persistence of contacts with ants. *Zoology* (Jena) 99 : 128-137.

Fiedler, K. & Maschwitz, U., 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.

Fiedler, K. & Maschwitz, U., 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.

Fiedler, K. & Saam, C., 1994. Does ant-attendance influence development in 5 European lycaenid butterfly species ? *Nota lepid.* 17 : 5-24.

Fiedler, K., Schurian, K. G. & Hahn, M., 1994. The life-history and myrmecophily of *Polyommatus candalus* (Herrich-Schäffer) from Turkey (Lep., Lycaenidae). *Linn. belg.* 14 : 315-332.

Godske, L., 1992. Aphids in nests of *Lasius flavus* F. in Denmark II : Population dynamics. *Ent. Meddr.* 60 : 21-26.

Henning, S. F., 1983. Chemical communication between lycaenid larvae (Lepidoptera : Lycaenidae) and ants (Hymenoptera : Formicidae). *J. Entomol. Soc. Sth. Afr.* 46 : 341-366.

Hölldobler, B. & Wilson, E. O., 1990. The ants. Harvard University Press, Cambridge/Mass.

Huxley, C. R. & Cutler, D. F. (Eds.), 1991. Ant-plant interactions. Oxford University Press, Oxford/New York/Tokyo.

Leimar, O. & Axén, A., 1993. Strategic behavior in an interspecific mutualism : interactions between lycaenid larvae and ants. *Anim. Behav.* 46 : 1177-1182.

Malicky, H., 1969. Versuch einer Analyse der ökologischen Beziehungen zwischen Lycaeniden (Lepidoptera) und Formiciden (Hymenoptera). *Tijdschr. Entomol.* 112 : 213-298.

MARTÍN CANO, J., 1984. Biología comparada de *Lampides boeticus* (L.), *Syntarucus pirithous* (L.) y *Polyommatus icarus* (Rot.) (Lep., Lycaenidae). *Graellsia* 40 : 163-193.

MASCHWITZ, U., WÜST, M. & SCHURIAN, K., 1975. Bläulingsraupen als Zuckerlieferanten für Ameisen. *Oecologia* 18 : 17-21.

PIERCE, N. E., 1987. The evolution and biogeography of associations between lycaenid butterflies and ants. *Oxford. Surv. evol. Biol.* 4 : 89-116.

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.

PONTIN, A. J., 1958. A preliminary note on the eating of aphids by ants of the genus *Lasius* (Hym., Formicidae). *Entomol. Monthly Mag.* 94 : 9-11.

PONTIN, A. J., 1978. The number and distribution of subterranean aphids and their exploitation by the ant *Lasius flavus* (Fabr.). *Ecol. Entomol.* 3 : 203-207.

SACHS, L., 1992. Angewandte Statistik. 7th edition. Springer Verlag, Berlin/Heidelberg/New York.

SCHURIAN, K. G., 1989. Revision der *Lysandra*-Gruppe des Genus *Polyommatus* Latr. (Lepidoptera : Lycaenidae). *Neue entomol. Nachr.* 24 : 1-181.

WAGNER, D., 1993. Species-specific effects of tending ants on the development of lycaenid butterfly larvae. *Oecologia* 96 : 276-281.

WAGNER, D., 1995. Pupation site choice of a North American lycaenid butterfly : the benefits of entering ant nests. *Ecol. Entomol.* 20 : 384-392.

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