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Ants benefit from attending facultatively myrmecophilous Lycaenidae caterpillars: evidence from a survival study

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Abstract Workers of three ant species (*Lasius niger*, *Lasius flavus*, *Myrmica rubra*) were caged in the laboratory together with caterpillars and pupae of five species of lycaenid butterflies. Mortality of ants was 3–5 times higher when the ants were confined with larvae lacking a dorsal nectar organ (*Lycaena phlaeas*, *Lycaena tityrus*) rather than with caterpillars which possess a nectar gland (*Aricia agestis*, *Polyommatus bellargus*, *P. icarus*). For all five species, ant survival was always lower at the pupal stage (where a nectar organ is always absent) than at the caterpillar stage and was largely equivalent for the butterfly species tested. The experimental data confirm earlier estimates that ants can derive nutritive benefits from tending facultatively myrmecophilous lycaenid caterpillars, even though these caterpillars produce nectar-like secretions at low rates.

Key words Butterflies · Ants · Mutualism · Myrmecophily

Introduction

Mutualistic interactions between different kinds of organisms are very common in nature (Boucher 1985). To qualify an interaction as mutualistic, benefits to both participants must be demonstrated (Cushman and Beattie 1991). This, however, often turns out to be experimentally difficult. The butterfly family Lycaenidae is a case in point. A large number of lycaenid species live in association with ants during part of their life cycle, mostly as larvae (Fiedler 1991a). The relationships between certain lycaenid species and their host ants are clearly parasitic (e.g. between the Palearctic butterfly genus *Maculinea* and ants of the genus *Myrmica*: Thomas et al. 1993), and other lycaenid-ant associations may be commensalic (Pierce and Young 1986). But most lycaenid-ant interactions are commonly believed to be mutualistic: larvae are protected by attendant ants which they appease and attract with nutrient rewards from specialized glands.

The majority of lycaenid species are facultative myrmecophiles (Pierce 1987; Fiedler 1991a). Facultative myrmecophiles can survive without tending ants, they are not specific to particular ant taxa, and their solitary larvae are normally tended by only one to five worker ants at any one time. Furthermore, their larval secretions appear to be more dilute in nutrients (Maschwitz et al. 1975; Wagner 1994). A number of lycaenid butterflies, however, are obligately and specifically bound to particular host ants (Fiedler 1991a). Those obligate myrmecophiles cannot survive without their ant partners (Pierce et al. 1987). They often deliver nutritive secretions to their ant guard at very high rates (e.g. Fiedler and Maschwitz 1989b), their dorsal nectar organ (DNO) secretions are rich in nutrients (Pierce 1989; Cushman et al. 1994), and large numbers of ants (10–15) are attracted to each individual caterpillar (Pierce et al. 1987; Cushman et al. 1994). This indicates that larvae of such obligate myrmecophiles constitute substantial food sources.

Most myrmecophilous lycaenid larvae possess a DNO located mediodorsally on the seventh abdominal segment. Secretions from this gland are rich in carbohydrates and/or amino acids (Maschwitz et al. 1975; Pierce 1983) and may contribute to the nourishment of ants (Fiedler and Maschwitz 1988). The importance of DNO secretions for the outcome of caterpillar-ant interactions has been experimentally demonstrated (e.g. Fiedler and Maschwitz 1989a). On the other hand, the larvae of a considerable number of lycaenid species never develop a DNO, and such larvae are usually not tended by ants in nature (Fiedler 1991a,b). The pupae of most lycaenid butterflies also lack a functional DNO (but see Hill 1993). In addition, larvae as well as pupae of lycaenids carry minute glandular structures like the pore cupola organs (Malicky 1969) or dendritic setae (Ballmer and Pratt 1992). At least in some lycaenids the pore cupolas secrete amino acids and therefore take part in the delivery of nutrient rewards to ants (Pierce 1983), but for

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most species the chemical nature of the secretions of these glands remains unknown. Furthermore, since pore cupolas and modified setae are ubiquitous in lycaenid immatures, their involvement in the nutrient flow from butterfly larvae to ants can be expected to be much less variable, whereas the presence or absence of a nectar gland can have pronounced consequences (e.g. Fiedler and Maschwitz 1989a).

Three types of benefits have already been shown to accrue to lycaenid butterflies through myrmecophily: the caterpillars are exempted from attacks by many ant species (Malicky 1969; DeVries 1991); the caterpillars are protected by their ant guard against other enemies (Atsatt 1981; Pierce and Mead 1981; Pierce and Easteal 1986; Pierce et al. 1987; DeVries 1991; but see Peterson 1993); and caterpillars may develop better when tended by ants (Fiedler and Hölldobler 1992; Wagner 1993; Cushman et al. 1994; Fiedler and Saam 1994; but see Robbins 1991; Baylis and Pierce 1992).

In contrast, nutritional benefits to the ants are less well documented. Pierce et al. (1987) showed that *Iridomyrmex* ants retrieve a substantial mass of food from trees inhabited by groups of caterpillars of the Australian obligate myrmecophile *Jalmenus evagoras*, and Nash (1989) found that colonies of *Iridomyrmex vicinus* ants grew better when they had access to *J. evagoras* immatures. With another Australian obligate myrmecophile, *Paralucia aurifera*, Cushman et al. (1994) demonstrated that survival of *Iridomyrmex anceps* ants caged with a caterpillar was better than among ants caged with a piece of host plant only. Because of their multiple specializations, however, these two obligate myrmecophiles may be quite atypical for the family Lycaenidae as a whole.

For facultatively myrmecophilous lycaenids only indirect estimates of the ants′ nutritional benefits are available, and these are based on experimental data on secretion rates, duration of larval development, and size and energy content of secretion droplets. Fiedler and Maschwitz (1988) thus estimated the cumulative lifetime volume of secretions passed to tending ants by caterpillars of the European *Polyommatus coridon* and concluded that individual caterpillars may contribute some 3.5–7 mg carbohydrates to the nourishment of ants over a period of 4 weeks. Recent studies on five additional Palearctic facultative myrmecophiles (Fiedler et al. 1994; Fiedler and Hagemann 1995; Burghardt and Fiedler in press) arrived at secretion volumes of 1–10 μl, which provide attendant ants with 0.15–1.5 mg carbohydrates in a period of 5–7 days. Comparison of these estimates with physiological data on ant metabolism (e.g. Peakin and Josens 1978; Nielsen 1986; Duncan and Lighton 1994) indicates that such small amounts of secretions from individual caterpillars may nevertheless suffice to cover the energetic requirement of small groups of ants for several days.

In the present study we quantified survival of ants caged with larvae of five European lycaenid species to obtain direct evidence as to whether caterpillars with a DNO provide a substantial energy resource for ant workers. As a form of control, we included species whose larvae, as well as the pupae, lack a DNO.

Material and methods

Butterfly species

We used three facultative myrmecophiles with a well-developed larval DNO (*Aricia agestis, Polyommatus bellargus, P. icarus*), and two species without a larval DNO (*Lycaena phlaeas, Lycaena tityrus*: Fiedler 1991a). Third and fourth instar larvae of *Aricia agestis, Polyommatus bellargus*, and *P. icarus* are unspecifically tended by a variety of ants, especially by *Lasius* and *Myrmica* species (Fiedler 1991b). Judging from field observations, the association with ants is closer in *A. agestis* and *P. bellargus* than in *P. icarus* (Emmet and Heath 1990; Thomas and Lewington 1991). As in many lycaenid species (Fiedler 1988), associations with ants also extend into the pupal period. Caterpillars of *Lycaena tityrus* and *Lycaena phlaeas* are not normally attended by ants, although weak unstable ant-associations can be induced in the laboratory (Fiedler 1991a).

Caterpillar rearing

Eggs were obtained from butterflies in a greenhouse culture. From the egg stage until butterfly eclosion, the immature stages were kept in an environmental chamber (constant temperature of 25°C, 16:8 hours L:D cycle). Caterpillars were reared in closed transparent plastic rearing vials (125 ml) lined with moist filter paper. The vials contained freshly cut inflorescences or foliage of the respective host plants in excess. We changed filter paper and food daily to reduce the risk of infections. The following host plants were used: *A. agestis* – *Geranium molle* leaves (Geraniaceae); *P. bellargus* – *Coronilla varia* leaves (Fabaceae); *P. icarus* – *Medicago sativa* inflorescences (Fabaceae); *Lycaena phlaeas, Lycaena tityrus* – *Rumex acetosa* leaves (Polygonaceae). The high humidity prevented rapid withering of the food and also ensured that the ants would not suffer from desiccation. All butterflies originated from northern Bavaria except part of the *P. bellargus* stock (from southern France). Details on the rearing procedure are given by Fiedler and Saam (1994).

Ants

Lasius niger, Lasius flavus and *Myrmica rubra* are common ants of grassland habitats in much of the Palearctic region, where they mainly occupy mesic to humid habitats. *L. niger* and *M. rubra* are omnivorous ants. They prey or scavenge on arthropods and collect homopteran honeydew or plant nectar (Kutter 1977; Seifert 1988). *L. flavus* ants are more specialized; they predominantly feed on honeydew of particular root aphids, but also prey on these trophobionts (Pontin 1978). All three ant species regularly attend lycaenid immatures in the field as well as in the laboratory (Fiedler 1991a,b). Ants were maintained in their original earth nests (two colonies of *L. flavus*) or in laboratory nests (plastic boxes with a bottom layer of plaster of Paris: various colonies of *L. niger*, one colony of *M. rubra*). The colonies of *L. flavus* and *M. rubra* had been excavated in northern Bavaria, the *L. niger* colonies had been raised from mated queens in the laboratory. Ants were fed honey-water and dead cut cockroaches as needed.

Experimental treatments

On the day the caterpillars moulted into the third instar (i.e. with the appearence of the DNO in the three myrmecophilous species), the larvae were individually placed in new rearing vials (125 ml, equipment as above) and randomly assigned to one of the following four ant treatments: caged with five worker ants of *Lasius flavus*; with two *L. flavus* ants; with two *Lasius niger* ants; or with one *M. rubra* ant. From that time onwards, each caterpillar (or later pupa) was constantly kept in association with ants in the environmental chamber until the adult butterfly eclosed. We transferred the caterpillars and ants into new clean rearing vials daily, supplying fresh food plants and removing the larval faeces. If ants died during the developmental period of the butterflies, they were replaced by nestmates in the course of daily inspections of all experimental lycaenid immatures. All lycaenids which died in the larval or pupal stages were discarded. In all, ant survival associated with 346 lycaenid individuals was analysed for this study.

Data recording and analysis

We separately noted the number of ants that died during the larval (third plus fourth instar) and pupal stage for each individual butterfly. We also recorded the time (in days) required to pass the larval and pupal stage (Fiedler and Saam 1994). From these data we calculated the rate at which ants had to be replaced (ants/day). This rate served as an estimate of ant mortality. Using two-tailed non-parametric statistics (Wilcoxon´s matched-pairs signed-ranks test, Mann-Whitney *U*-test, Kruskal-Wallis *H*-test: Sachs 1992) we compared ant mortality between conspecific larvae and pupae, or between species. Frequency distributions were analysed using χ^2 procedures. Throughout the text, data are given as means ± SD.

Results

Ant survival with lycaenid larvae

In general, ants survived significantly better when caged with larvae which have a DNO (Table 1). In the experimental series with five *Lasius flavus* workers per lycaenid immature, ant survival was equal among the two *Lycaena* species without DNO ($U = 244$, $z = 0.357$, $P > 0.7$), as well as among the three myrmecophilous species ($H_{2\ \mathrm{df}} = 0.535$, $P > 0.7$). The difference between mortality at larvae with (0.079 ± 0.129 ants/day) or without a DNO (0.468 ± 0.366 ants/day) was highly significant ($U = 703$, $z = 4.309$, $P < 0.0001$).

In experiments with two *Lasius flavus* workers per caterpillar, there was a clear-cut separation between species with or without a larval DNO. Myrmecophiles with DNO (0.056 ± 0.091 ants/day, $H_{2\ \mathrm{df}} = 1.897$, $P > 0.35$) as well as non-myrmecophiles without DNO (0.170 ± 0.182 ants/day, $U = 129$, $z = 0.89$, $P > 0.35$) were statistically homogeneous, whereas the difference between both groups was distinct ($U = 673.5$, $z = 3.236$, $P < 0.002$). Hence, depending on the exact species combination, *Lasius flavus* ants survived on average 3–6 times better when they were caged with lycaenid larvae with a DNO.

When two *Lasius niger* workers were confined with lycaenid immatures, the three species with larval DNO (*A. agestis, P. bellargus, P. icarus*) again supported ant

Table 1 Daily mortality of ants (replaced ants/day, means ± SD) at single larvae and pupae of five European lycaenid butterfly species (*P/L* ratio of mean mortality at pupae versus mean mortality at larvae). Statistics: *z* values of Wilcoxon matched-pairs signed-ranks test, significance relates to two-tailed test

	Five *Lasius flavus*	Two *Lasius flavus*	Two *Lasius niger*	One *Myrmica rubra*
Aricia agestis				
Larvae	0.334 ± 0.569	0.051 ± 0.104	0.052 ± 0.084	0.000 ± 0.000
Pupae	0.821 ± 0.988	0.412 ± 0.364	0.329 ± 0.134	0.096 ± 0.076
	(n=25)	(n=28)	(n=14)	(n=6)
Statistics	$z = 3.823$***	$z = 4.143$***	$z = 3.170$**	$z = 1.826^{+}$
P/L	2.46	8.08	6.33	>1
Polyommatus bellargus				
Larvae	0.076 ± 0.088	0.070 ± 0.085	0.026 ± 0.049	0.000 ± 0.000
Pupae	1.477 ± 0.701	0.511 ± 0.255	0.153 ± 0.131	0.137 ± 0.088
	($n = 14$)	($n = 13$)	($n = 11$)	($n = 8$)
Statistics	$z = 3.296$***	$z = 3.040$**	$z = 3.823$***	$z = 2.380$*
P/L	19.43	7.30	5.89	>10
Polyommatus icarus				
Larvae	0.081 ± 0.105	0.053 ± 0.084	0.008 ± 0.025	0.026 ± 0.041
Pupae	0.995 ± 0.502	0.336 ± 0.211	0.283 ± 0.156	0.071 ± 0.059
	($n = 19$)	($n = 16$)	($n = 19$)	($n = 13$)
Statistics	z = 3.724***	z = 3.408***	z = 3.823***	z = 2.134*
P/L	12.28	6.34	35.38	2.7
Lycaena phlaeas				
Larvae	0.491 ± 0.402	0.157 ± 0.204	0.049 ± 0.094	0.036 ± 0.063
Pupae	0.940 ± 0.638	0.377 ± 0.254	0.229 ± 0.173	0.105 ± 0.090
	(n = 26)	(n = 24)	(n = 26)	(n = 17)
Statistics	z = 2.745**	z = 3.136**	z = 3.893***	z = 2.551*
P/L	1.91	2.40	4.67	2.9
Lycaena tityrus				
Larvae	0.439 ± 0.324	0.194 ± 0.148	0.105 ± 0.098	0.070 ± 0.06963
Pupae	0.742 ± 0.464	0.278 ± 0.233	0.172 ± 0.154	0.096 ± 0.096
	(n = 20)	(n = 13)	(n = 17)	(n = 17)
Statistics	z = 2.627**	z = 1.019	z = 1.525	z = 1.161
P/L	1.69	1.48	1.64	1.37

(+$P<0.1$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$)

Table 2 Mortality of ants with single larvae of five European lycaenid species. Given are numbers of experimental replicates where all original ants survived until pupation versus those replicates in which at least one ant died prior to pupation. Statistics are χ^2 values of contingency tests (first value with 4 *df*: comparison between all five species; second value with 1 *df*: comparison of larvae with or without a dorsal nectar organ (DNO)

No. and species of ant	All ants survived	At least one ant died	Statistics
Five *Lasius flavus*			
Aricia agestis	18	7	
Polyommatus bellargus	6	8	
P. icarus	10	39	$\chi^2_{4\,df} = 16.12$**
Lycaena phlaeas	6	20	
Lycaena tityrus	5	15	
Larvae with DNO	34	24	$\chi^2 = 12.59$***
Larvae without DNO	11	35	
Two *Lasius flavus*			
Aricia agestis	21	7	
Polyommatus bellargus	6	7	
P. icarus	10	6	$\chi^2_{4\,df} = 10.91$*
Lycaena phlaeas	12	12	
Lycaena tityrus	3	10	
Larvae with DNO	37	21	$\chi^2 = 4.93$*
Larvae without DNO	15	22	
Two *Lasius niger*			
Aricia agestis	9	5	
Polyommatus bellargus	8	3	
P. icarus	17	2	$\chi^2_{4\,df} = 15.06$**
Lycaena phlaeas	18	8	
Lycaena tityrus	5	12	
Larvae with DNO	34	10	$\chi^2 = 5.45$*
Larvae without DNO	23	20	
One *Myrmica rubra*			
Aricia agestis	6	0	
Polyommatus bellargus	8	0	
P. icarus	9	4	$\chi^2_{4\,df} = 12.29$*
Lycaena phlaeas	11	5	
Lycaena tityrus	7	10	
Larvae with DNO	23	5	$\chi^2 = 5.10$*
Larvae without DNO	7	10	

(* $P<0.05$; ** $P<0.01$; *** $P<0.001$)

survival significantly better than the two *Lycaena* species without DNO ($U = 695.5$, $z = 2.509$, $P < 0.02$). Among the species without DNO, ant survival was higher with the fast developing *Lycaena phlaeas* than with the more slowly growing *Lycaena tityrus* ($U = 137$, $z = 2.268$, $P < 0.03$).

In the series with one *M. rubra* ant per lycaenid, there was again a perfect separation between larvae with (0.013 ± 0.025 ants/day, $H_{2\,df} = 4.846$, $P = 0.089$) or without a DNO (0.053 ± 0.070 ants/day, $U = 104$, $z = 1.537$, $P > 0.12$). Both groups were statistically homogeneous and differed significantly from another ($U = 308$, $z = 2.673$, $P < 0.01$). A single *M. rubra* worker ant survived on average 4 times better if caged with a lycaenid larva with a DNO.

A similar pattern emerges when one compares the ratio of experiments where all ants survived until a caterpillar eventually pupated (Table 2). In experiments with five *Lasius flavus* workers tending *Lycaena* larvae without DNO, all ants survived throughout the third and fourth larval instar in less than 25% of all trials, while with caterpillars possessing a DNO complete survival of the original ants occurred in 58.6% of the experiments. Parallel results were obtained in the other three series of experiments (two *Lasius flavus* ants: 63.8% complete survival with DNO, 40.5% without DNO; two *Lasius niger* ants: 77.3% complete survival with DNO, 53.5% without DNO; one *M. rubra* worker: 85.2% complete survival with DNO, 54.5% without DNO).

Complete survival of the ant guard occurred less frequently with the slowly growing *Lycaena tityrus* than with the fast developing congeneric *Lycaena phlaeas*, but this difference was statistically significant only in the series with two *Lasius niger* ants per larva ($\chi^2_{1\,df} = 5.048$, $P < 0.025$). Among the three lycaenid species with larval DNO, no significant differences occurred with respect to the frequency of complete survival of the ant guard until pupation ($\chi^2_{2\,df} < 5.06$, $P > 0.075$ in all four experimental series), despite pronounced differences in the duration of larval development.

Ant survival with lycaenid pupae

In all four experimental series, survival of ants was lower with the pupae of five lycaenid species than with their caterpillars (Table 1). This was significant throughout except for three test series with *Lycaena tityrus*. The pupa-to-caterpillar ratio of mean ant mortality was higher than 5.5 in 10 out of 12 experimental series involving species with a larval DNO (exceptions were five *Lasius flavus* with *A. agestis* and one *M. rubra* with *P. icarus*), whereas this ratio was below 3.0 in 7 of 8 series involving lycaenids without a larval DNO (two *Lasius niger* ants at *Lycaena phlaeas* provided the only exception).

Ant survival with lycaenid pupae differed only slightly between the five butterfly species tested. No significant differences were observed in experiments with either two *Lasius flavus* ants ($H_{4\ \mathrm{df}} = 6.352$, $P > 0.17$) or with one *M. rubra* worker ($H_{4\ \mathrm{df}} = 3.984$, $P > 0.4$). In trials with five *Lasius flavus* ants, an exceptionally high mortality occurred with *P. bellargus* pupae (comparison with *P. icarus*: $U = 191$, $z = 2.114$, $P < 0.04$), while the remaining four lycaenid species formed a homogeneous group ($H_{3\ \mathrm{df}} = 3.998$, $P > 0.25$). With two *Lasius niger* ants, ant survival was poorest with *A. agestis* and highest with *P. bellargus* pupae, but there was no clear interspecific pattern.

Discussion

Our experimental data revealed that (1) ants survive much better if they tend lycaenid caterpillars with a DNO, and (2) that ant survival decreases with lycaenid pupae with only marginal differences between the butterfly species tested. The first result demonstrates that the secretions of facultatively myrmecophilous lycaenid caterpillars in fact contribute to the nourishment of ants, even though secretion rates of *P. icarus* or *A. agestis* larvae are in the range of only 5–20 droplets/hour (Burghardt and Fiedler in press). The ants in our experiments had access only to four potential food sources: plant sap oozing from feeding damage, caterpillar faeces, secretions from the DNO (in myrmecophilous caterpillars only), and secretions of other glands (pore cupola organs, dendritic setae). Since food plants and caterpillar frass were equally present in all cases, and because all five species tested possess pore cupolas, the better survival of ants at caterpillars of the three myrmecophiles must be attributed to the secretory products of their DNOs.

We observed no significant differences in the ant mortality per day between the three myrmecophilous species *A. agestis, P. bellargus* and *P. icarus*. This suggests that in equal time periods secretions of these caterpillars are roughly equivalent as food resources. However, the duration of larval development differed considerably between these species. *A. agestis* larvae took 6–9 days to pass the third and fourth instar, *P. icarus* needed 10–12 days and *P. bellargus* 18–23 days (Fiedler and Saam 1994). As a consequence, total mortality tended to accumulate in species with longer larval development. For example, *Lasius flavus* worker ants more often survived completely with the fast growing *A. agestis* than with the more slowly developing *Polyommatus* species. Overall, mean ant mortality per day at caterpillars without a DNO was 3–5 times higher than at larvae with DNO. Confining ants with the rapidly growing *Lycaena phlaeas* (final two instars lasted 7–11 days) turned out to be less stressful for ants than experiments with the congenor *Lycaena tityrus*, whose larvae required 10–18 days to pass the third and fourth instar (Fiedler and Saam 1994).

Thirty-eight of 153 *Lasius flavus* ants (24.8%) used in experiments with two ants per caterpillar died while caged with caterpillars with a DNO during the larval stage, whereas 66 of 140 ants (47.1%) did not survive with larvae without a DNO. Similar figures were obtained in the other three experimental series (five *Lasius flavus* ants: 26.8 versus 49.5%; two *Lasius niger* ants: 13.7 versus. 30.0%; one *M. rubra*: 12.9 versus 37.0%). These data further confirm that ants obtain little, if any energetic benefits from interacting with caterpillars lacking a DNO. As a corollary, ants usually do not recruit nestmates to such caterpillars (Fiedler and Maschwitz 1989a), and associations between caterpillars without DNO and ants induced in the laboratory tend to disintegrate within a few hours (Fiedler 1991a).

This low rentability of caterpillars without DNO as food resource is the most plausible reason why such larvae are almost never found together with tending ants in nature, although some of the communication signals seen in myrmecophilous species are present (Malicky 1969; Ballmer and Pratt 1992). Because we did not include a series of replicates where ants were kept without any caterpillars, we can at present not decide whether the secretions from ant-organs such as pore cupolas or dendritic setae make a substantial contribution to the nourishment of worker ants or whether they are merely involved in caterpillar-ant communication.

The importance of DNO secretions in lycaenid-ant mutualisms is further emphasized by the prominent increase in ant mortality which occurred with the three myrmecophilous species after pupation. As with most other lycaenids (but see Hill 1993), the pupae of our study species do not have a nectar organ. Hence, with the disappearance of the DNO, ant mortality approached the figures observed for species which do not have a nectar gland. Within the pupal stage, differences between the lycaenid species were marginal, suggesting that pupal secretions of all five species represent equally constraining food resources. Survival of ants tended to be particularly low with pupae of *P. bellargus*. In the experimental series with five *L. flavus* ants this effect was even significant. This can be explained by the relatively long pupal period of *P. bellargus* (11–13 days: Fiedler and Saam 1994). Ants which had already been caged with *P. bellargus* caterpillars for a larval period of 18–23 days faced a greater risk of starvation in experiments with this butterfly species.

Ant survival also decreased during the pupal stage of non-myrmecophilous *Lycaena* species, though not as strongly as in the three myrmecophilous lycaenid species. This could be explained as an accumulation of mortality when ants had been separated from their home colonies for extended periods. In addition, ants may have fed upon plant sap oozing from feeding damage or upon caterpillar faeces. Both behaviours are known from ants interacting with lycaenid immatures (e.g. Fiedler 1991a; Seufert and Fiedler, unpublished manuscript) and could well contribute to the nourishment of ants when caged with feeding larvae. In experiments with pupae, neither host plant parts nor larval faeces were available, thus further constraining food availability.

Like the work of Cushman et al. (1994), our survival experiments have three important restrictions. First, we assessed benefits to the ants not on the colony level, but for groups of foragers. Although natural selection works on the colony rather than individual level among social insects (e.g. Heinze et al. 1994), it nevertheless seems safe to conclude that energetic benefits to workers should finally translate into fitness gains for the whole colony. Second, to ensure constant tending levels throughout the larval and pupal development, we caged the ant workers with lycaenid immatures in isolation from their home colony. Hence, the ants experienced no foraging costs. On the other hand, mortality may have accumulated to unnaturally high levels because of the extended isolation of the foragers. We can presently not estimate how large this experimental error is, but it was certainly equal in all experimental series. Third, with our method we could not detect any benefits that ant brood may derive from lycaenid secretions. The metabolism of ant workers is mainly based on carbohydrates (Duncan and Lighton 1994), but ant larvae also require amino acids for development and growth. Hence, even though worker ants derive little benefits from tending lycaenid pupae, the pupal secretions, which according to Pierce (1983) contain amino acids, might well be important for brood-raising.

The data presented here are the first direct evidence that secretions of facultatively myrmecophilous lycaenid caterpillars contribute to the nourishment of ants. In particular, our data emphasize the importance of the larval nectar gland as a source of nutrient rewards. Therefore, the mutualistic nature of facultative lycaenid-ant associations, heavily challenged by Malicky (1969), gains additional strength.

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