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

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 commensal (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 larval 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
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 Eastal 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 lycaenid species 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 Jøsens 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 trophobions (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 appearance of the DNO in the three myrmecophilous species), the larvae were individually placed in new rearing vials (125 ml,