

Strategic behaviour in an interspecific mutualism: interactions between lycaenid larvae and ants

OLOF LEIMAR & ANNKRISTIN H. AXÉN*

Department of Zoology, Stockholm University, S-106 91 Stockholm, Sweden

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Abstract. Interspecific mutualism often involves partners trading different services. If it is costly to provide a service, there is a conflict of interest, and mutualism cannot be evolutionarily stable unless partners react to each other's contributions. An individual might also be influenced by its need for the services offered, being more willing to contribute when the need is greater. In mutualistic interactions between lycaenid butterfly larvae and ants, the larvae secrete nutritious droplets from a specialized gland and, in return, the ants protect the larvae from attacks by parasitoids and predators. Here, two aspects of the interactions between larvae of the common blue, *Polyommatus icarus*, and ants were studied experimentally. First, larval reaction to ant attendance was investigated by giving varying numbers of ants, *Lasius flavus*, access to a larva. The amount of larval secretion increased with the level of ant attendance, but at high levels no further increase occurred. Second, the effect of variation in a larva's need for protection was studied in an experiment where ants, *Tetramorium caespitum*, had free access to a larva. Exposing the larva to a simulated attack resulted in increased secretion and increased ant attendance. The effect on secretion was stronger; the number of droplets per attending ant was higher after an attack, making the interaction more favourable for the ants.

Mutualistic interactions between species can often be viewed as a trade of different kinds of services. In cases where at least one participant pays a cost to provide something that solely benefits a partner, there is clearly a conflict of interest. The benefit of the interaction to this individual comes only from services provided by the partner. Without being subject to some form of social control, i.e. punishment and reward delivered by the partner, the individual would benefit most by not providing anything. This implies that strategic reactions to the contributions of others are necessary for the evolutionary stability of mutualistic behaviour in these cases (cf. Axelrod & Hamilton 1981).

In a relationship where the efforts towards cooperation can be varied gradually, it is clear that a partner's contribution could affect an individual's own behaviour. In addition to this, an individual could, depending on the situation, vary in its need for the partner's contribution. Such variation in need is likely to affect the individual's own contribution. When mutualistic partners contribute different kinds of commodities, one ought to expect

that principles analogous to the law of supply and demand determine the rates at which these commodities are traded. Thus, an individual in great need of the services rendered by a partner ought to be particularly willing to contribute (cf. Noë et al. 1991).

In the butterfly family Lycaenidae probably more than 50% of all species associate in some way with ants (Malicky 1969; Pierce 1987). The associations can be parasitic or commensal, but most relationships seem to be mutualistic (Malicky 1969; Cottrell 1984). The strength of these lycaenid–ant associations is highly variable, and ranges from loosely facultative unspecific relationships to strictly obligate associations (Fiedler 1991). In the mutualistic relationships, lycaenid larvae secrete a nutritious liquid from a specialized gland, the dorsal nectar organ, on the dorsum of the seventh abdominal segment (Newcomer 1912). The liquid, which contains carbohydrates and amino acids (Maschwitz et al. 1975; Pierce 1989), is collected by the attending ants. The ants use their antennae to palpate the posterior part of the larva, around the dorsal nectar organ, and this stimulates the larva to release a droplet. In return, the ants protect the

*To whom all correspondence should be sent.

larvae from attacks by parasitoids and predators (Pierce & Mead 1981; Pierce & Eastal 1986; Pierce et al. 1987). On the segment posterior to the one with the dorsal nectar organ, lycaenid larvae have a pair of eversible tentacles. Their function is unclear, but it is believed that they exude substances that both attract ants and evoke alarm behaviour (Henning 1983; Fiedler & Maschwitz 1987; Fiedler 1991).

In cases with strong interactions, release of droplets is costly for the larvae (Pierce et al. 1987). Tended individuals pupate at a lower weight than their untended counterparts, and the adults eclosing from these pupae are lighter and smaller. In facultative myrmecophilous species, the evidence for the cost of myrmecophily is somewhat less distinct (Robbins 1991). If release of droplets is costly, a larva would profit if it could get attendance without providing any secretion. As a consequence, ant reaction to larval secretion is necessary for the evolutionary stability of the relationship. Such reactions have been observed by Fiedler & Maschwitz (1989).

If ants react to larval secretion, it would be in a larva's best interest to change the amount secreted in response to changes in attendance. By secreting only a little at low levels of attendance and more at higher levels, the larva could be surrounded by more ants than would be needed simply to remove the secretion, and thus the larva might gain better protection. To investigate this matter, we conducted an experiment in which we measured changes in the rate of larval secretion as a response to manipulated variation in the level of ant attendance. We found that the larvae reacted to variation in ant attendance.

One of the factors that would affect a lycaenid larva's need for ant protection is the probability of enemy attacks. Thus, if a larva receives information of an increased attack risk, it ought to increase its willingness to provide secretion. To test this, we exposed larvae to simulated predator attacks and found that the larvae increased their contribution.

METHODS

Study Animals

Larvae of *Polyommatus icarus* (Rottemburg, 1775) [Lycaenidae: Polyommataini], from eggs laid by wild-caught females, were reared in climatic chambers (at 23°C:15°C and 21 h daylength) on

plants of *Medicago sativa*. *Polyommatus icarus* larvae have, as far as is known, facultative and unspecific associations with several ant species from different ant genera and subfamilies (Fiedler 1991).

Two ant species, *Lasius flavus* (Fabricius, 1781) [Formicidae: Formicinae] and *Tetramorium caespitum* (Linnaeus, 1758) [Formicidae: Myrmicinae], were kept in earth nests (at 23°C and 19 h daylength). The colonies were fed daily with a standard ant diet (Bhatkar & Whitcomb 1970) supplemented with fruit flies. We used these two species because they are easy to hold in captivity and have been shown to tend larvae of *P. icarus* under laboratory conditions (Fiedler 1990).

Larval Reaction to Ant Attendance

Each of 26 fourth instar larvae went through three treatments, with different numbers of attending ants: treatment 1, a single ant had access to the larva; treatment 2, two ants had access; treatment 3, many ants had access.

To induce tending, foraging *L. flavus* ants were allowed free access to larvae and pupae in a petri dish, connected to the ant's nest by means of a bridge. In treatments 1 and 2, the larva was kept on an *M. sativa* leaf in a 5 ml dish. When the treatment started, one or two ants, occupied with tending, were taken from the petri dish to the dish with the larva. In treatment 3, the larva was placed in the petri dish, where ants were already tending other larvae. The larvae were able to eat both during and between trials.

Each treatment was 20 min long and started when the first ant began to court the larva. The interaction was observed through a stereo microscope, and every 10th s the number of ants that touched the larva was counted to give information about the number of ants directly attending to the larva. The numbers of, and the times for, secreted droplets and tentacle eversions were noted. All three treatments were carried out the same day, in random order, for each larva, and with at least 1 h between trials.

Larval Reaction to Simulated Attacks

Each of 21 larvae went through two treatments. In one treatment we simulated an attack by a predator or parasitoid by lightly pinching the larva with a pair of tweezers, and in the other the larva

Table I. Effect of increased ant attendance on larval behaviour ($\bar{X} \pm SE$)

	Treatments			P*
	1	2	3	
Droplets per 20 min	1.35 ± 0.28	2.5 ± 0.33	2.08 ± 0.25	0.017
Tentacle eversions per 20 min	23.9 ± 5.3	31.0 ± 6.4	21.8 ± 4.0	0.8
Attending ants	0.81 ± 0.02	1.53 ± 0.05	3.83 ± 0.27	<0.001

Each *P. icarus* larva ($N=26$) went through three treatments: 1, a single *L. flavus* ant had access to the larva; 2, two ants had access; 3, more than two ants had access.

*Kruskal-Wallis one-way analysis by ranks.

Table II. Larval response ($\bar{X} \pm SE$) to simulated attacks

	Simulated attack	Control	P*
Droplets per 5 min	2.19 ± 0.26	1.14 ± 0.35	0.0092
Tentacle eversions per 5 min	19.2 ± 3.9	3.8 ± 1.2	0.0005

Each of 21 *P. icarus* larvae, attended by *T. caespitum* ants, went through two treatments. In one of these the larva was pinched to simulate a predator attack. In the control, the larva was left undisturbed.

*Wilcoxon matched-pairs signed-ranks test.

was left undisturbed. Pinching the larva was an attempt to imitate a failed attack, where a predator or parasitoid first grasps the larva and then drops it. In the laboratory we have observed parasitic wasps dropping *P. icarus* larvae before managing to oviposit.

During the experiment a fourth instar *P. icarus* larva was placed in a petri dish connected to a *T. caespitum* nest, so that the ants had free access to it. After 30 and 60 min we watched the interactions for 5 min and counted the numbers of droplets and tentacle eversions. The number of attending ants was observed just before the onset of each 5-min period and then once every minute. Before one of these two 5-min periods, randomly chosen, the larva was pinched to simulate a predator attack. The other 5-min period was used as a control.

RESULTS

Larval Reaction to Ant Attendance

The number of attending ants affected larval behaviour. When the number of ants having access increased from one to two, the larvae almost doubled the number of droplets secreted ($z=2.52$, $N=26$, $P=0.01$, Wilcoxon matched-pairs signed-

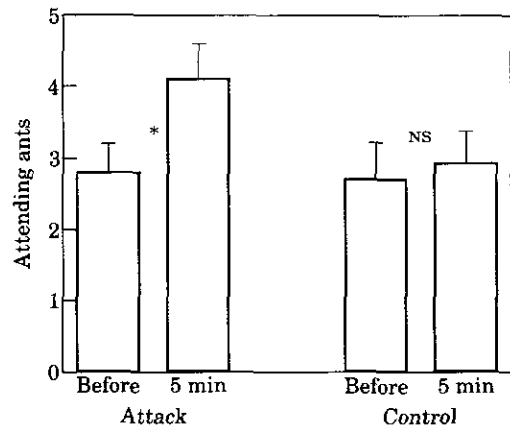


Figure 1. The level of ant *T. caespitum*, attendance ($\bar{X} \pm SE$) before and after a simulated attack on a *P. icarus* larva. The number of attending ants was measured immediately before the attack and during the following 5 min. In the control the larva was left undisturbed. * $P < 0.01$, $N = 21$.

ranks test). When the number of ants increased even more, there was no further increase in larval droplet delivery (Table I). Number of tentacle eversions did not differ significantly between treatments (Table I). We found no significant correlation between larval weight and number of

droplets delivered ($r_s = -0.13$, $N = 26$, $P = 0.51$) or ant attendance ($r_s = 0.32$, $N = 26$, $P = 0.11$; weights varied from 24 to 92 mg).

Larval Reaction to Simulated Attacks

The larvae gave significantly more droplets after a simulated attack. They almost doubled the number of droplets delivered during the 5-min period immediately following the attack (Table II). Only one larva did not secrete any droplets, in any of the two treatments. The rate of tentacle eversions differed even more between the treatments, increasing by about a factor of five after the attack (Table II).

The ants responded to the increased effort of the larvae by increasing the degree of attendance (Fig. 1). In the control treatment there was no difference between the number of attending ants immediately before ($\bar{X} \pm SE = 2.71 \pm 0.50$) and during ($\bar{X} \pm SE = 2.93 \pm 0.44$) the 5-min period ($z = 0.68$, $N = 21$, $P = 0.49$, Wilcoxon matched-pairs signed-ranks test). In the treatment with a simulated attack there were significantly more ants attending to the larva during the 5-min period after the attack ($\bar{X} \pm SE = 4.11 \pm 0.50$) than immediately before it ($\bar{X} \pm SE = 2.81 \pm 0.39$; $z = 3.08$, $N = 21$, $P = 0.002$). To see how long the effect of an attack lasted, we compared the 11 larvae where the control treatment was conducted first with the 10 larvae where the control was conducted 30 min after the simulated attack. The control period values of larval secretion, tentacle eversions and ant attendance did not differ significantly between these two groups (respectively, $P = 0.57$, 0.23 , 0.62 , $N_1 = 11$ and $N_2 = 10$ in all cases, Mann-Whitney U -tests). Thus, after 30 min the simulated attack no longer influenced the interaction.

Although both larval secretion and ant attendance increased during the 5-min period following an attack, the effect on secretion was stronger. The average ratio (droplets during control)/(droplets after attack) was 0.59. This is significantly different from the ratio (ants during control)/(ants after attack) which equalled 0.82 ($z = 2.45$, $N = 20$, $P = 0.014$, Wilcoxon matched-pairs signed-ranks test). Thus, the number of droplets per attending ant increased after an attack.

DISCUSSION

We have shown that, as the level of ant attendance increases, the rate of secretion of *P. icarus* larvae

first increases and then levels off (Table I). The rate of secretion also seems to be affected by a larva's estimate of the need for protection, whether from parasitoids and predators or perhaps from ant attacks, almost doubling after a simulated attack. Tentacle eversions and ant attendance also increased after an attack (Table II).

The two ant species used in these experiments have not been observed attending *P. icarus* larvae in the field, although *T. caespitum*, at least, probably does so. Our main aim in this study was to test the effect of different degrees of ant attendance and stress on larval behaviour. The choice of ant species should not affect the outcome of these experiments, as long as the ants attend the larvae. It may be more difficult to interpret the response of ants to increased larval effort in the second experiment. It is not known if *T. caespitum* responds to tentacle eversions of *P. icarus* (Fiedler & Maschwitz 1987; Fiedler 1991), but in our experiment they responded by increased attendance to either secretions or tentacle eversions, or both.

As mentioned, evolutionary stability of the mutualistic relationship between lycaenids and ants requires strategic reactions to the partner's contribution. To induce a recruitment response in the ants, a larva should perhaps be generous initially, but later on in the interaction it ought to regulate its contribution according to the level of attendance. No detailed predictions concerning such a strategy are available. The pattern we found might be typical, with larval secretion first increasing and then levelling off, or perhaps even decreasing at high levels of attendance. With several ants attending, the protective value of an additional ant is likely to be small, so that a larva should give little additional secretion for the increased attendance.

It is known that ants regulate the level of attendance depending on larval secretion. Fiedler & Maschwitz (1989) have shown that ants attend larvae less if they do not provide any secretion. Other observations indicate that both the amount and the quality of larval secretion influence the degree of ant attendance (Fiedler 1990; Baylis & Pierce 1991). Henning (1983) suggested that one of the functions of the larval tentacle eversions was to attract ants. A larva could then gain at least a short-term increase in attendance by signalling with its tentacles. This is consistent with our observation that both tentacle eversions and ant attendance increased after a simulated attack. Henning's other suggestion, that tentacle eversions evoke alarm

behaviour in the ants, is also consistent with this observation of increased tentacle eversions, since the larva had reason to fear another attack.

When a lycaenid larva secretes liquid to gain ant attendance, it increases its survival at the expense of growth. The optimum trade-off between growth and survival for the larva depends on how much survival increases through attendance. Variation in the need for protection might be an important factor explaining differences between lycaenid species in the strength of ant association. As indicated by our experiment, a larva could also trade off growth for survival in the short term, depending on its information about the need for protection.

In mutualistic species interactions, it is probably common that the level of investment by an individual is influenced by the need for the partner's services. In the ant-lycaenid relationship, an ant colony's need for larval secretion depends on the amount and quality of that secretion in relation to other food sources available. That the degree of ant attendance is affected by larval quality (manipulated through diet) has been shown by Fiedler (1990) and Baylis & Pierce (1991). It is quite likely that alternative food sources influence the number of ants attending a larva. Nash (1989) showed that the number of ants per larva decreased when an ant colony had access to several larvae.

When an individual increases its contribution because of a greater need for the services provided by the partner, this partner is likely to respond by also increasing its contribution. One might, however, expect that the transaction should become more favourable for the partner, who now has a more valuable commodity to trade. In the simulated attack experiment we found evidence for this kind of phenomenon. When the larva was in greater need of protection, the ants obtained secreted droplets more cheaply.

Taken at face value, this observation would indicate that the ants are using an efficient strategy when dealing with lycaenids. Such an interpretation must, however, be made with considerable caution. While it is clear that lycaenids have adaptations for dealing with ants, there is in fact little evidence that ants have adaptations for dealing with lycaenid larvae (cf. Nash 1989). It is possible that lycaenids use chemical manipulation to exploit the tending behaviour of ants towards other trophobionts (Pierce 1987; Nash 1989).

In a study of the iterated Prisoner's Dilemma game, Axelrod & Hamilton (1981) proposed that

cooperation, in the form of a strategy called Tit for Tat, could be evolutionarily stable. A player of this strategy cooperates in the first interaction, and in following interactions repeats the partner's previous behaviour. Thus, Tit for Tat entails a form of social control.

Following Axelrod & Hamilton's study, Tit-for-Tat-like behaviour has been looked for in cases where animals cooperate. That individuals react to the partner's behaviour has been shown in several studies (Fischer 1980; Milinski 1987; Dugatkin 1988, 1991; Sella 1988; Milinski et al. 1990). These observations indicate the occurrence of social control, but leave open the exact nature of the strategies used.

In a Prisoner's Dilemma, players have only two behaviour patterns at their disposal, cooperate or defect. A drawback of this simplification is that it does not allow for gradual variation in cooperative behaviour, which is probably quite common in nature. The simplification of the iterated Prisoner's Dilemma game has further consequences. As mentioned, many factors apart from the partner's behaviour may influence an individual's own behaviour, but strategies like Tit for Tat do not take such factors into account. For predator inspection in sticklebacks, *Gasterosteus aculeatus*, Külling & Milinski (1992) have shown that an individual will approach a predator more closely when together with a larger partner. They also showed that the larger of a pair is more often attacked, so that an individual is more protected by the presence of a large partner. This means that the quality of a partner, in this case in the form of size, influences an individual's behaviour. Similarly, our study showed that the need for the partner, which can be seen as a measure of quality, had an effect. Phenomena such as these clearly show that cooperative behaviour is more complex than can be described by a simple strategy like Tit for Tat.

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