

Signalling in a mutualistic interaction

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Abstract. The costs and benefits for partners in interspecific, mutualistic relationships can vary from one interaction to the next, or change with time during an interaction, which might influence the mutualistic contributions. Such flexible behavioural responses could be important in regulating the interaction. Partners could also have special adaptations for the purpose of influencing each other. An individual might, for instance, signal its need for the services provided by the partner. In interactions between lycaenid butterfly larvae and ants, the larvae deliver nutritious droplets from a specialized gland and, in return, are protected from enemy attacks by the ants. Lycaenid larvae have several ant-related adaptations, one being a pair of eversible tentacular organs. In this study, the relationships between tentacle display, the delivery of droplets and ant attendance were investigated in a number of experiments, where the interaction between larvae of *Polyommatus icarus* and either *Lasius niger* or *L. flavus* ants was manipulated. High rates of both droplet delivery and tentacle display were found at the first contact with ants, after an interruption in attendance and, for low levels of attendance, after a simulated enemy attack. Under steady state conditions, displays were most frequent at low levels of attendance, but droplet delivery had a maximum at intermediate levels. Manipulation of a larva's ability to use its tentacles also showed that ants respond to tentacle display by increasing their attendance. Larval behaviour was interpreted as aimed at regulating the number of attending ants.

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The fitness consequences of mutualistic interactions are often subject to many kinds of variability. The costs and benefits of exchanging services are likely to depend both on the states of either partner and on the surrounding situation (Bronstein 1994), all of which may differ from one interaction to the next, or fluctuate over time in a single interaction. Factors such as the quality of the partner, the availability of alternative partners or resources, and the need for the services provided will influence the degree to which an interaction is beneficial. A partner's quality may vary with size, age or condition (Bronstein 1994). Other species with which the mutualists interact, such as host plants, predators and other potential mutualists, can also affect the interaction in a way that varies both over the short term and between habitats and seasons. If a partner provides protection, the service may not be needed at all if the

density of a major predator is very low. For instance, Cushman & Whitham (1989) found that ant protection had no detectable effect on membracid survival in years when predators were scarce. Similarly, if a partner provides food, the availability and quality of alternative food sources will influence the value of the service (Way 1954; Cushman & Addicott 1989).

These kinds of variability in costs and benefits, together with transfer of information about the variability between partners, may have an important influence on the nature of mutualistic relationships. An individual's decision to participate in or to terminate an exchange ought to depend on its expected payoff. Also, if mutualistic efforts can be varied gradually, an individual ought to regulate its contribution according to both the level of services provided by the partner and the need for these services.

An individual's actual contribution inevitably reveals aspects of its abilities and needs. One might, however, also expect specific adaptations for the transfer of information. High quality

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individuals could benefit from signalling their high ability to provide services, thereby increasing their chances of getting a partner or of getting a higher quality partner. Similarly, an individual experiencing an increased need ought to inform a partner about its increased willingness to cooperate.

It is often possible to classify mutualists as either hosts or visitors (Cushman & Beattie 1991), where hosts are relatively less mobile than visitors. In these situations, hosts may use signals to attract visitors, as is the case for animal-pollinated flowers, as well as to induce the visitors to initiate an interaction. In addition to this, we suggest that signals may also be used to regulate an ongoing interaction.

In the experiments reported here, we investigate the contributions of lycaenid butterfly larvae to their tending ants in a variety of circumstances, and relate variation in these contributions to a particular larval behaviour, referred to as tentacle signalling.

Roughly half of the species in the butterfly family Lycaenidae form more or less close associations with ants during their larval stage (Malicky 1969; Pierce 1987). The majority of these interactions seem to be mutualistic, ranging from obligate to facultative relationships (Malicky 1969; Cottrell 1984; Fiedler 1991). The basis of the interaction is the trade of two commodities: protection and food. Ants protect their lycaenid partners against predators and parasitoids (Pierce & Mead 1981; Pierce & Eastal 1986) and in return they receive nutritious droplets secreted from a specialized gland (Pierce et al. 1987).

Several factors influence the costs and benefits for the participants in lycaenid-ant interactions. A larva's ability to secrete droplets depends, for instance, on host plant quality (Fiedler 1990; Baylis & Pierce 1991). The need for the mutualistic service, and thus perhaps the willingness to participate, is likely to vary for both lycaenids and ants. Since the ants provide protection, the current risk of enemy attack will determine the value of this service. An ant colony's need for lycaenid secretions will largely depend on the current availability of alternative food sources, such as aphids and extrafloral nectaries. In trophobionts, both intra- and interspecific competition for ant attendance have been found (Buckley 1983; Cushman & Addicott 1989).

Both participants are able to vary their contributions. Larvae can alter the rate of secretion according to the level of ant attendance (Leimar & Axén 1993), and larval age, quality and productivity affect how ants allocate workers between different larvae (Pierce et al. 1987; Fiedler 1990; Baylis & Pierce 1991). Fiedler & Maschwitz (1989) showed that lycaenid butterfly larvae experimentally prevented from releasing secretion were less able to elicit ant recruitment behaviour.

Lycaenid larvae possess several traits that facilitate ant-association, and that potentially could reveal information about a larva's motivational state. The tentacles are one such example: a simulated enemy attack leads to a greatly increased rate of tentacle display (Leimar & Axén 1993). Another example might be the substrate-borne vibrations produced by some lycaenid larvae (DeVries 1990; Schurian & Fiedler 1991). Such behaviour could provide useful information to the ants, but might also contain elements of lycaenid manipulation of ants.

Our aim in this study was to investigate the function of the tentacular signal. In a series of experiments we manipulated larval conditions, and thus motivational state, and observed the response in secretion and tentacle display. We manipulated the larval motivational state both through variation in ant attendance, with the intention of giving a larva different information about the willingness of ants to invest in tending, and through variation in the risk of enemy attack. We also manipulated a larva's ability to use its tentacles, to see whether ants reacted to the signal.

GENERAL METHODS

Study Animals

In all experiments we used larvae of the facultatively myrmecophilous butterfly *Polyommatus icarus* (Rottemburg, 1775) (Lycaenidae: Polyommatainae). The larvae were raised from eggs laid by wild-caught females, and reared on plants of *Medicago sativa*, at 25°C and in a 21:3 h light:dark regime.

Polyommatus icarus larvae form relationships with several different ant species (Fiedler 1991) and, like many other lycaenids, have a number of traits of importance in their relationship with ants

(Fiedler 1991), two of which concern us here. First the dorsal nectar organ, on the seventh abdominal segment, secretes a liquid, in the form of discrete droplets, which are collected by ants. The chemical composition of the secretion is known only for a few lycaenid species; in the case of *P. icarus*, it contains several carbohydrates (Maschwitz et al. 1975). The droplets are released only in response to ant stimulation, as when ants use their antennae to palpate a larva on the area around the gland. Second, on the eighth abdominal segment, and flanking the dorsal nectar organ, there are two eversible tentacles. Although their exact function has not been conclusively determined, worker ants of several, but not all (Fiedler 1991), species react strongly to tentacle eversions. Apparently, some form of chemical communication is involved. On eversion of the tentacles, nearby ants become very alert or alarmed (Henning 1983; Fiedler & Maschwitz 1987), and sometimes perform characteristic 'excited runs', or attempt to grasp an everted tentacle with the mandibles. In *P. icarus*, a tentacle display is a discrete event, lasting around 1 s or less.

Ant colonies of two species of the genus *Lasius* were kept in earth nests in the laboratory at about 22°C. *Lasius niger* (Linné, 1758) (Formicidae: Formicinae) tends aphids as well as several lycaenid species, including *P. icarus*, in the field (Fiedler 1991). *Lasius flavus* (Fabricius, 1781) (Formicidae: Formicinae) collects honeydew from root-living aphids. Owing to their subterranean life-style they probably rarely tend lycaenids in the field, but they easily form relationships with lycaenid larvae in the laboratory, of a kind similar to that of *L. niger*.

Experimental Set-up

We performed five experiments, examining different aspects of the interaction between ants and *P. icarus* larvae. All had the same general set-up, with one or several foraging arenas where interactions took place and were observed through a stereo microscope. The foraging arenas were connected to an ant colony by means of plastic tubes. A foraging arena consisted of an open 200 ml plastic container with Fluon coated sides, which prevented ants from leaving except through the plastic tube. We could control the number of ants in each arena by closing the entrance with a plug and in some cases removing excess ants with a

small brush. Food, in the form of sugar water and chopped maggots, was provided only in these arenas. During experiments the ant food was removed.

We set up an interaction by removing the ants from an arena and placing a fourth instar larva on a *M. sativa* leaf. The larva was allowed to feed during a trial to make it more stationary and easy to observe in the stereo microscope. We left the larva undisturbed for 10 min and then gave the ants access to the arena by removing the plug. During observation periods the ants that touched the larva were counted every 10th second. We also noted the number of, and the times for, secreted droplets and tentacle eversions. Values of these quantities are reported as $\bar{X} \pm \text{SE}$.

Statistical Methods

Since the number of droplets secreted and the number of tentacle eversions during an observation period were not normally distributed, we used non-parametric statistics for the analysis of these quantities. All comparisons performed were matched: either the same larva in different situations or differently treated larvae taking part in the same trial. We performed paired comparisons with the Wilcoxon matched-pairs signed-ranks test (abbreviated Wilcoxon test), and for similar comparisons over more than two situations we used the Friedman test. The average number of attending ants during an observation period was more closely normally distributed and was analysed with parametric statistics. In all cases, we used two-tailed tests.

EXPERIMENTS

Larval Reaction at First Contact with Ants

Methods

Our aim was to investigate whether a larva's initial rate of droplet delivery differed from the rate later on during the relationship, and if so, whether tentacle eversions followed a similar pattern. We observed 21 *P. icarus* larvae for 10 min immediately following their first contact with *L. niger* ants and then again for 10 min starting 60 min after first contact. The ants had free access to the larva during a trial.

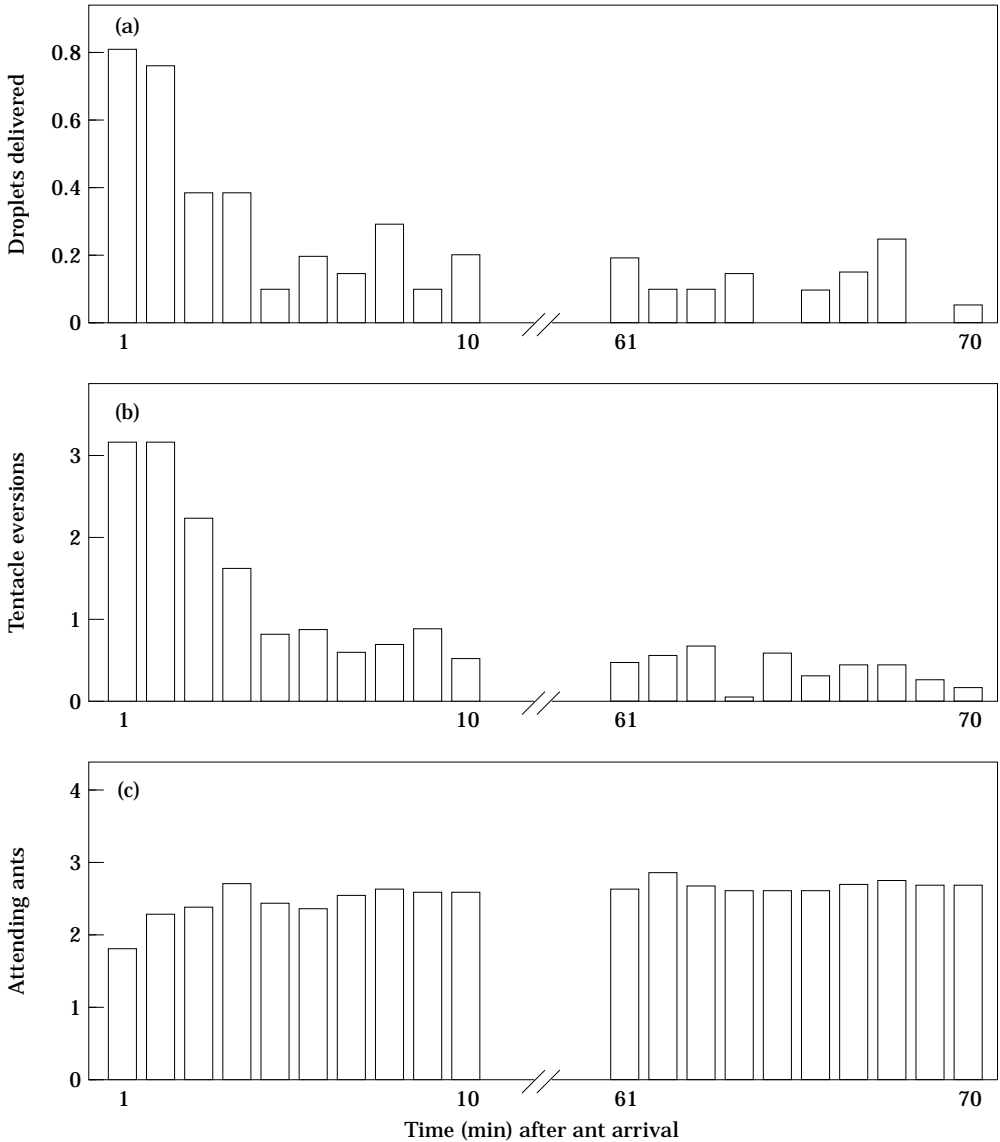


Figure 1. Larval behaviour immediately following the first contact with *L. niger* ants, and later on during the interaction: (a) mean number of droplets per min and (b) mean number of tentacle eversions per min for 21 larvae. (c) Average number of attending ants during the same period.

Results

At first contact, the larvae both released more droplets and everted the tentacles more frequently (Fig. 1) than they did later on during the interaction. About three times as many droplets were released during the first 10 min of contact (3.33 ± 0.44) as in the 10-min period after 1 h of

interaction (1.05 ± 0.22 ; Wilcoxon test: $T=0$, $N=21$, $P<0.001$). Similarly, the number of tentacle eversions was almost four times higher at the beginning of the interaction (14.57 ± 2.12) than later on (3.86 ± 1.35 ; Wilcoxon test: $T=4$, $N=21$, $P<0.001$). The rates of droplet delivery and tentacle eversion varied more or less in parallel, and the rates started to decrease a few

minutes after first contact (Fig. 1). Both droplet delivery ($T=6$, $N=21$, $P<0.01$) and rate of tentacle eversions ($T=1$, $N=21$, $P<0.001$) were higher in the first 5 min after contact than in the following 5 min. The average number of attending ants seemed to build up to a steady level over the first few minutes of the interaction (Fig. 1). However, there was no significant difference between the average number of attending ants during each of the two 10-min periods (2.41 ± 0.19 and 2.62 ± 0.19 , respectively; paired-sample t -test $t=1.13$, $df=20$, $P=0.27$).

Larval Reaction to Temporary Absence of Ants

Methods

In the field, lycaenid larvae are likely to encounter temporal fluctuations in the number of attending ants. In this experiment we investigated larval behaviour before and after a shorter or longer interruption of an otherwise continuous ant attendance. We used three treatments, differing in the duration of ant absence, with 13 larvae being subjected to a given treatment (39 larvae in total). In the first treatment we prevented the ants from tending the larva for 60 min, and the second treatment had a similar 5-min interruption. The third treatment was a control where the interaction was left undisturbed. We carried out the treatments simultaneously in three separate arenas, connected to one ant colony (*L. flavus*), in order to control for variation in the state of the colony, which can cause variation in the level of attendance. One larva was placed in each arena and the different treatments were assigned at random to the arenas. The ants had free access to the arenas, except during the interruptions. For each interaction there were three separate 10-min observation periods. The first observation period came 60 min after the start of the interaction, and was followed by the interruption of ant attendance (first and second treatments). The second observation period started on the return of ants to the larva, or, for the control, after 60 min of undisturbed interaction. Finally, the third period started 60 min after the end of the second period.

Results

Figure 2 shows droplet delivery and tentacle eversion for the different treatments and

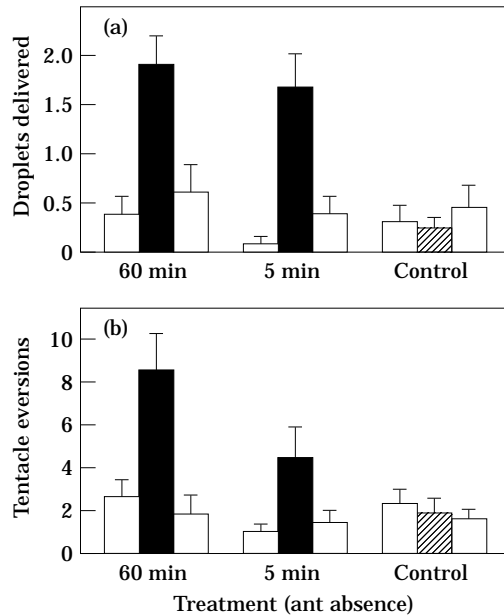


Figure 2. Larval behaviour before and after an interruption of an otherwise continuous ant attendance. In two treatments the ants (*L. flavus*) were removed for either 60 min ($N=13$) or 5 min ($N=13$), and then reintroduced to the larva. The number ($\bar{X} \pm SE$) of (a) droplets and (b) tentacle eversions were measured during 10 min: before, immediately after (■), and 1 h after an interruption in the interaction. In a control treatment ($N=13$), the interaction was left undisturbed and was observed with 1-h intervals. Hatched bars in the control treatment correspond to the same observation period as black bars in the treatments with ant absence.

observation periods. For the treatment with a 60-min interruption, the rate of droplet delivery differed between the three observation periods, and the same was true for the rate of tentacle eversion (Friedman test: droplets: $\chi^2=16.63$, $df=2$, $P<0.001$; tentacles: $\chi^2=15.32$, $df=2$, $P<0.001$; $N=13$). Similarly, for the 5-min interruption treatment both droplet delivery and tentacle eversion differed between the observation periods (Friedman test: droplets: $\chi^2=17.69$, $df=2$, $P<0.001$; tentacles: $\chi^2=8.14$, $df=2$, $P=0.017$; $N=13$), whereas the control treatment did not show any such differences (Friedman test: droplets: $\chi^2=0.56$, $df=2$, $P=0.76$; tentacles: $\chi^2=0.18$, $df=2$, $P=0.91$; $N=13$).

In addition, comparing the three treatment groups at a fixed observation period, and matching by trial, showed that both droplet delivery and

tentacle eversion differed between the treatment groups for the second period, immediately after the return of ants (Friedman test: droplets: $\chi^2=15.24$, $df=2$, $P<0.001$; tentacles: $\chi^2=8.52$, $df=2$, $P=0.014$; $N=13$). In contrast, there were no such differences for either the first period (Friedman test; droplets: $\chi^2=2.33$, $df=2$, $P=0.31$; tentacles: $\chi^2=2.59$, $df=2$, $P=0.27$; $N=13$) or the third period (Friedman test: droplets: $\chi^2=0.50$, $df=2$, $P=0.78$; tentacles: $\chi^2=1.68$, $df=2$, $P=0.43$; $N=13$).

The statistical significances were caused by a higher rate of both droplet delivery and tentacle eversion immediately following the return of ants (Fig. 2). The duration of ant absence (60 min versus 5 min) did not influence the number of droplets delivered on return of the ants (Wilcoxon test: $T=28.5$, $N=13$, $P=0.69$), but there was a non-significant tendency towards a less pronounced increase in the number of tentacle eversions following the shorter, 5-min absence (Wilcoxon test: $T=16.5$, $N=13$, $P=0.08$).

Larval Reaction to Level of Ant Attendance

Methods

To induce more ants to attend it, a larva might deliver more droplets when the number of attending ants is high (Leimar & Axén 1993). However, since the need for additional ants ought to decrease with increasing numbers of already attending ants, it is conceivable that delivery might decrease at high levels of attendance. For the same reason, the rate of tentacle eversion might also be lower with more ants attending.

To investigate this, we exposed 16 larvae to different levels of ant (*L. flavus*) attendance and measured the number of droplets delivered and the rate of tentacle eversion. Each larva went through four treatments, with either one, two, four, or many (10 or more) attending ants. For the first three treatments, we controlled the number of ants by closing the entrance with a plug and in some cases removing excess ants with a small brush. For the treatment with many ants we kept the colony hunger level high and gave the ants free access to a larva. When the desired number of ants had been present for 10 min we observed the interaction for 15 min. All treatments for a given larva were carried out the same day and in a randomized order.

As in the other experiments, the times for droplets and tentacle displays were recorded to the nearest 10 s. For the current experiment, we used these data to see whether tentacle eversions tended to occur near in time to the delivery of a droplet. A given tentacle eversion was considered near in time to droplet delivery if there was a droplet within ± 35 s of the time of eversion; otherwise the eversion was considered far in time from droplet delivery.

Results

The number of attending ants affected larval behaviour (Fig. 3). Both droplet delivery and tentacle eversion differed between the treatments (Friedman test: droplets: $\chi^2=17.94$, $df=3$, $P<0.001$; tentacles: $\chi^2=31.52$, $df=3$, $P<0.001$; $N=16$). As a function of the level of ant attendance, the rate of droplet delivery first increased but then decreased at high levels of attendance (Fig. 3). In contrast with the previous two experiments, the rate of tentacle eversion did not vary entirely in parallel with droplet delivery. Instead, the larvae used the tentacles most frequently when only one ant was attending, and progressively less as more ants attended. With many ants, the rate dropped almost to zero.

Although this experiment indicates that tentacle eversion cannot be viewed simply as directly coupled to droplet delivery, there is still the possibility that the time sequence of eversions is correlated with the delivery of droplets. However, an analysis of the time sequence of larval behaviour showed that larvae did not have a higher (or a lower) rate of tentacle eversion around the time a droplet was delivered (Table I). Thus, for steady state conditions, the two types of behaviour appear to be independent of each other.

The Effect of Ant Attendance and Simulated Attacks

Methods

Polyommatus icarus larvae respond to simulated enemy attacks by increasing the rates of both droplet delivery and tentacle eversion (Leimar & Axén 1993). The behaviour was interpreted as a reaction to a change in a larva's estimate of the need for protection. However, if a larva already experiences a high level of ant attendance, little

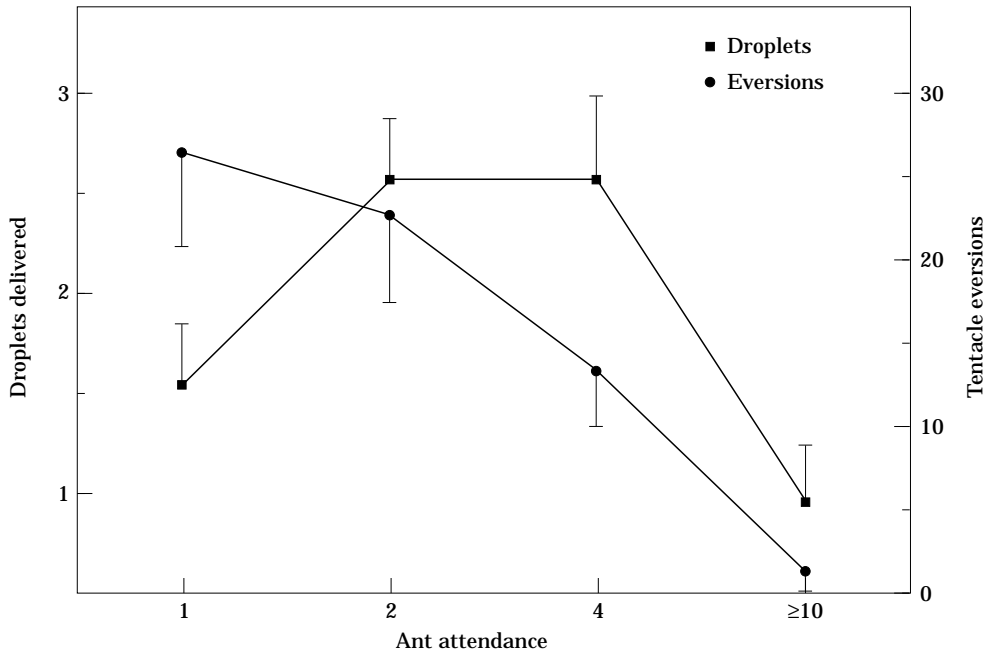


Figure 3. Larval response to different levels of ant attendance: number ($\bar{X} \pm SE$) of droplets and tentacle eversions during 15 min. Each larva ($N=16$) went through four different treatments, with either one, two, four, or at least 10 attending *L. flavus* ants. The actual numbers of tending ants in the different treatments were 0.92 ± 0.02 , 1.72 ± 0.05 , 3.46 ± 0.07 and >10 .

Table 1. Tentacle displays occurring near in time (± 35 s) versus far in time from a droplet delivery, based on all trials in the experiment with manipulation of the number of attending ants

Ant attendance	Near/far	Tentacle displays		P^\dagger
		Observed	Expected*	
1	Near	61	61.99	0.89
	Far	360	359.01	
2	Near	84	80.50	0.66
	Far	275	278.50	
4	Near	59	55.40	0.56
	Far	174	177.60	
≥10	Near	2	2.80	0.60
	Far	16	15.20	

*Expected values assume a uniform rate of tentacle display within each trial.

†Level of significance in a chi-squared goodness of fit test.

additional protection can be gained, and the response to simulated attacks might be less pronounced.

To investigate the interaction between the level of attendance and the risk of enemy attack, each

of 28 larvae was attacked twice, once when attended by two (*L. flavus*) ants and once when attended by 10 or more ants. As in the experiment by Leimar & Axén (1993), we simulated an attack by lightly pinching the larva with a pair of tweezers, with the intention of imitating a situation where a parasitoid grasps the larva and then drops it before being able to oviposit. For a given larva, the two attack treatments (high versus low attendance) were performed in a random order. At the start of a trial, either two or many ants were given access to a larva. After 30 min we observed the interaction for 5 min, then pinched the larva lightly, and observed the ensuing interaction for 5 min. After this second observation, the number of ants was changed and, after an additional 30 min, there were again two 5-min observation periods with an intervening simulated attack.

Results

For low levels of ant attendance the larvae responded to a simulated attack by increasing

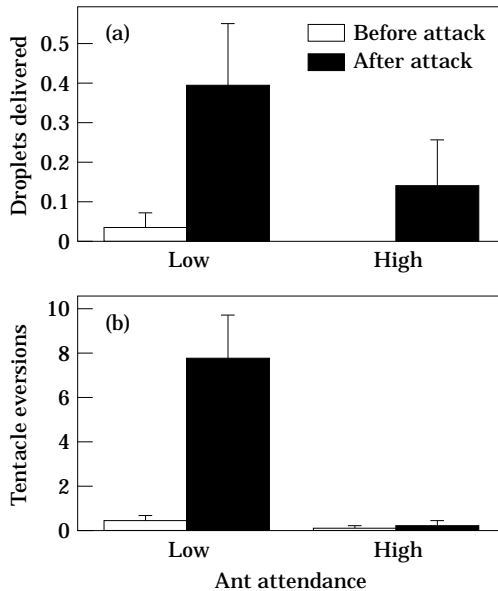


Figure 4. The effect of ant (*L. flavus*) attendance on larval reaction to simulated attacks. Each larva ($N=28$) was attacked twice, once at a low and once at a high level of ant attendance. The number ($\bar{X} \pm \text{SE}$) of (a) droplets and (b) tentacle eversions was measured during two 5-min periods, immediately before and after a simulated attack.

the rates of both droplet delivery and tentacle eversion (Wilcoxon test: droplets: $T=4.5$, $N=28$, $P=0.03$; tentacles: $T=0$, $N=28$, $P<0.001$; Fig. 4). For high levels of attendance the rates were low both before and after the simulated attack. Only two larvae released any droplets (this occurred after attack) and only one larva showed any tentacle display (twice before and six times after the attack), resulting in too many ties to perform a statistical test.

Ideally, one would want to use parametric statistics to test the attack risk by ant attendance interaction, but this cannot be justified with the current data. Instead, for each larva and attendance treatment, we computed the difference, after attack minus before attack, in droplet delivery and tentacle eversion, and compared these differences between low and high attendance. The attack-induced increase in droplet delivery was not significantly higher at low ant attendance, but there was a highly significant effect of ant attendance on the increase in tentacle eversion (Wilcoxon test: droplets: $T=12$, $N=28$, $P=0.37$;

tentacles: $T=0$, $N=28$, $P<0.001$). Thus, the simulated attack had a stronger effect on larval behaviour at a low number of attending ants.

Ant Response to Tentacle Display

Methods

The previous four experiments have been concerned with larval behavioural responses, in the form of droplet delivery and tentacle eversion, in a variety of circumstances. To examine the response of ants (*L. flavus*) to tentacle displays, and thus the importance for larvae of performing this behaviour, we manipulated a larva's ability to use its tentacles. Half of 44 larvae had their tentacles covered with a water-based Tipp-ex fluid, which prevented eversion. The larvae were tested in pairs, with one intact and one treated larva tested at the same time, but in different arenas. We measured the level of ant attendance during three 5-min observation periods, the first starting at a larva's first contact with ants, the second after 30 min of undisturbed interaction, and finally the third immediately after a simulated attack.

Results

Figure 5 shows the levels of ant attendance for intact and treated larvae. Comparing the total average number of attending ants over all three observation periods showed that larvae without functional tentacles were not able to recruit as high levels of ant attendance as intact larvae (paired t -test: $t=3.40$, $df=21$, $P=0.003$). Furthermore, a repeated measures ANOVA, with trial as observation unit and tentacle treatment and observation period as repeated measures factors, resulted in a significant tentacle treatment by observation period interaction ($F_{2,42}=5.00$, $P=0.01$), so that ant attendance levels varied differently between observation periods for the two tentacle treatments (cf. Fig. 5). Finally, a post-hoc test of ant attendance following the simulated attack showed that larvae with intact tentacles had more attending ants than those with covered tentacles (Tukey HSD test: $P<0.001$). Thus, by using tentacle displays a larva can attract more ants than would otherwise be the case and, in particular, can regulate the number of attending ants in response to short-term changes in the perceived risk of enemy attack.

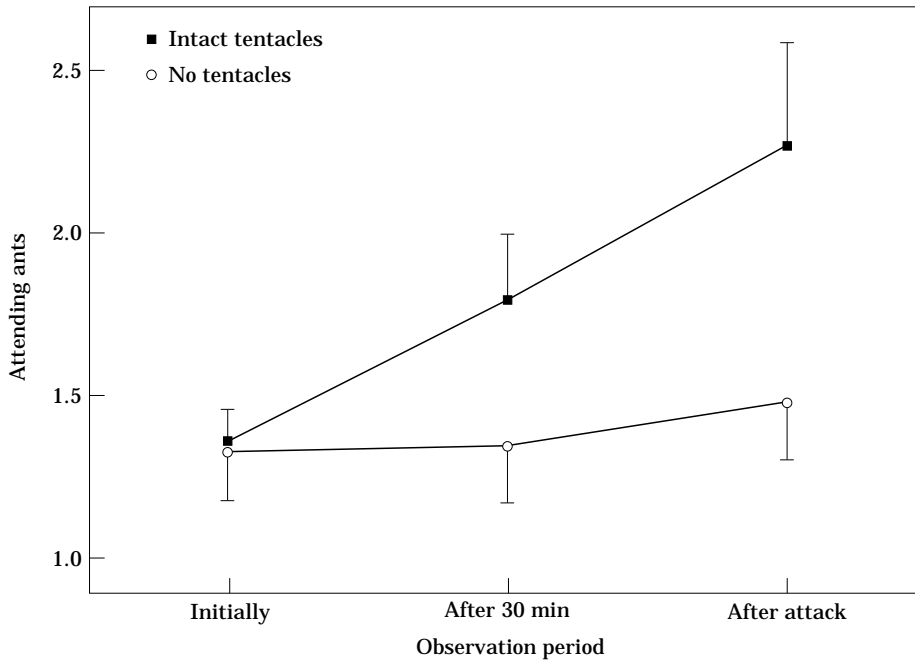


Figure 5. Level of ant (*L. flavus*) attendance towards larvae with or without functional tentacles ($N=22$ in both treatments). The number of attending ants ($\bar{X} \pm \text{SE}$) was measured during three different 5-min periods: at the start of the interaction, after 30 min, and after a simulated attack.

Larval behaviour followed the expected pattern. Control larvae had high rates of both droplet delivery and tentacle eversion initially and after the attack, and lower rates in the observation period prior to the attack (Friedman test: droplets: $\chi^2=7.60$, $df=2$, $P=0.02$; tentacles: $\chi^2=17.03$, $df=2$, $P<0.001$; $N=22$), and the rate of droplet delivery for treated larvae varied in a similar way (Friedman test: $\chi^2=16.62$, $df=2$, $P<0.001$, $N=22$). There were no significant differences in the total number of droplets delivered by control and treated larvae (Wilcoxon test: $T=57$, $N=22$, $P=0.21$).

DISCUSSION

Polyommatus icarus larvae were quite flexible in their ant-related behaviour. The rates of droplet delivery and tentacle display were affected both by ant attendance and the risk of enemy attack, and these two influences interacted in determining larval response. The interaction between ants and larvae also had a dynamic character; behavioural rates were adjusted over periods of a few minutes

or less. A consideration of this variation in larval behaviour ought to give some insight into its function. Furthermore, since larval secretions are collected by ants, it seems reasonable that a colony derives at least some nutritional benefit from the delivery of droplets. Such nutritional benefits have been shown in other lycaenid-ant relationships (Pierce et al. 1987; Fiedler & Maschwitz 1988). Assuming this to be the case, the relationship between droplet delivery and tentacle display might indicate whether ants could derive useful information from the signalling behaviour of a larva.

Interpretation of Tentacle Display

If we first consider immediate causation, one possibility would be that tentacle display is a direct response to the level of ant stimulation, but this is clearly not the case. Although the behaviour is performed almost exclusively in the presence of ants (lightly pinching a larva can sometimes produce display when no ants are present), a high intensity of ant stimulation seems rather to have a negative effect (Fig. 3). Similarly,

the delivery of droplets shows a more complex dependence on ant stimulation. From the point of view of larval motivation, most of our results would be consistent with the idea that both types of behaviour are controlled in parallel by the same motivational system (Figs 1, 2 and 4). However, the response to different levels of ant attendance (Fig. 3) does not fit so well with this idea; comparing low versus moderate attendance levels, droplet delivery and tentacle display changed in opposite directions. In addition, over very short time scales the two types of behaviour appear independent of each other (Table I), showing that display cannot be regarded as simply 'announcing' the delivery of a droplet.

As a more consistent interpretation of our experiments, we suggest that larvae use tentacle display as a means to regulate the number of attending ants. A number of circumstances point in this direction. High rates of display occurred when a larva might have been in need of more ants or, at least, would have had a good reason to avoid or fear a reduction in the level of attendance, such as when the degree of ant interest in the larva was uncertain (Figs 1 and 2), the level of attendance low (Fig. 3), or when the need for protection was high (Fig. 4). On the other hand, for very high levels of attendance a larva ought not to need more ants, not even when there is a high risk of enemy attack, and the rate of display is almost zero (Figs 3 and 4). Furthermore, ants will be rewarded with secretions if they respond to intense tentacle display by keeping up or increasing their level of attendance, and, finally, ants did respond with increased attendance to tentacle display (Fig. 5). This interpretation agrees with the suggestion that one function of the tentacles is to attract ants (Claassens & Dickson 1977; Henning 1983).

Since ants are rewarded with droplets if they respond to high rates of tentacle display and can avoid allocating an excessively high number of workers to a larva if they reduce their interest when the rate of display is low, the signal potentially contains useful information for the ants. Although the signal contains information about larval motivation, our experiments have not demonstrated that ants use this information to their own advantage. Also, even if the response of worker ants to the signal would make them more efficient at extracting secretions from a larva, or better able to protect it from harm, it could still be

the case that workers would do more good for their colony by foraging elsewhere.

The ability to manipulate ants is definitely present within the Lycaenidae. Larvae of some species, such as those of the genus *Maculinea*, induce ants to carry them into the nest (Elmes et al. 1991). Once inside, the larva starts to prey on the ant brood (Cottrell 1984; Elmes et al. 1991), so in these cases the lycaenid is entirely parasitic. For the more common and seemingly mutualistic associations, as with *P. icarus*, a more subtle form of manipulation may be present. If the substances released from the tentacles are mimics of ant alarm-pheromones, as suggested by Henning (1983), the selection pressure on ants from excessive tending of lycaenid larvae might be too small in comparison with the importance of this intra-colony communication system.

Since ants respond with increased attendance to tentacle display (Fig. 5), we must ask why a larva does not always signal at the highest possible rate, in order to maximize the number of tending ants. Eversion of the tentacles and production of the released substances must cost some energy, but perhaps not very much. If energetic costs prevent a larva from displaying except when in great need, the behaviour could be interpreted as handicap signalling (Grafen 1990). Other factors may, however, be more important. A very high number of attending ants could be disadvantageous for a larva, for instance by making it more conspicuous to predators, such as birds, against which the ants are often of little use. Lycaenid larvae tend to be highly cryptic, and, to the human eye, are difficult to spot except when accompanied by ants. Regulating ant numbers according to circumstances might thus be beneficial for a larva. Finally, and perhaps most importantly, the ant-lycaenid interaction is a dynamic process where some learning on the part of the ants is likely to operate. Ants respond most strongly, with 'excited' behaviour, to tentacle eversion at the start of an interaction, and later appear to habituate to the signal. By conserving the signal for when it is really needed, such as when an enemy attacks or the larva shifts location and wants the ants to come along, the larva maintains its ability to influence ant behaviour.

In addition to tentacle display, some lycaenids, including *P. icarus*, produce substrate-borne sounds (DeVries 1990; Schurian & Fiedler 1991), but the effect of these sounds on ants is unknown.

DeVries (1988, 1990) investigated the signalling behaviour of riodinid butterfly larvae. The riodinid sound-producing vibratory papillae appear to regulate the number of attending ants (DeVries 1988, 1990).

Experimental Situations

Our first experiment investigated the initial reaction of larvae to ant presence, and showed elevated rates of behaviour during the first few minutes of ant contact (Fig. 1). A similar effect was produced by an interruption in attendance (Fig. 2). The high number of droplets initially could be because a larva has stored up excess secretion when no ants were present, but the response to the different durations of ant absence argues against this possibility. The same number of droplets was delivered after a 5-min absence as after a 60-min absence, and the total number of droplets during the 10-min observation period following the brief absence was about four times higher than would be the case under steady state conditions, making it unlikely that these droplets were stored up during the 5-min break in attendance.

The high initial rate of secretion appears instead to be a means by which the larva attempts to get the interaction going, by inducing a recruitment response in the ants, and similarly to resume it after an interruption. In cooperative interactions between mobile partners, one should expect the initial phase to differ from the remainder. For symmetric situations where both partners are mobile, a 'slow start' with initial suspiciousness can be a method to avoid exploitation (Enquist & Leimar 1993). For a host-visitor situation, the host must attract a visitor and outcompete other activities of interest to the visitor. In particular, for a lycaenid larva a choosy strategy, where some ants are rejected in the hope of more profitable visits of workers, perhaps from some other colony, appears unlikely to be successful. Nevertheless, the visitors could exploit too much initial generosity, by quickly moving from one host to the next. The effect of the brief ant absence in our experiment suggests that attending ants could do better by implementing an occasional interruption in attendance.

A slightly different way of viewing the initial phase comes from noting that the partners have little information about each other at this point.

The ants do not know the productivity of the particular larva and the larva does not know the current foraging and nutritional situation for the ant colony. One could thus regard the initial larval investment as an exploratory effort to gain information about the level of ant interest. An interruption in ant attendance might have a similar effect of increasing larval uncertainty about the level of ant interest.

Larval reaction to the level of ant attendance (Fig. 3) resulted in a similar pattern of droplet delivery as previously observed (Leimar & Axén 1993). In the current experiment, we included a treatment with very high numbers of attending ants, to see whether this would lead to a lower rate of delivery. The high attendance (around 10 ants) was achieved by manipulating the nutritional status of the colony. Comparison with the lower levels of attendance, under free access, depicted in Fig. 5, shows the potential influence of colony state on the interaction. As many as 10 ants is more than has been observed for *P. icarus* larvae under field conditions and, as mentioned, might represent a problem for the larva, in which case the low rate of secretion could be an attempt to reduce the number of tending ants. From the ants' point of view, it would be suboptimal to allocate workers beyond the point of maximum secretion rate. In general, the levelling off of the rate of delivery with attendance (Fig. 3) agrees with what should be expected for an exchange obeying the law of supply and demand: a larva pays less for additional ants when it is already rather well protected.

In the experiment by Leimar & Axén (1993), there was no significant effect of more attending ants on the rate of tentacle eversions, whereas here we found a decrease (Fig. 3). A possible reason for the difference lies in the smaller variance in the number of attending ants, both over time and between trials, achieved with the method of regulating ant numbers in the current experiment. Considering the experiment with a temporary ant absence (Fig. 2), increased rates of display as a response to temporal variability in attendance seem fairly likely.

As previously shown (Leimar & Axén 1993), *P. icarus* larvae respond to a simulated enemy attack with increased rates of delivery and display. Here we found this response, at least with regard to tentacle display, to be inhibited by high levels of attendance (Fig. 4). This lends support to the

interpretation of larval behaviour as being aimed at regulating ant numbers. Coming back to the issue of the function of tentacle display, Fiedler & Maschwitz (1987) proposed that the seeming alarm reaction induced could make ants more efficient at detecting and averting an enemy (DeVries (1988) proposed a similar function for the anterior tentacle organs of riodinids). The effect appears quite likely, but one might expect the benefit of greater ant activity to be important also with many attending ants. In this sense the almost complete absence of larval tentacle response to a simulated attack (Fig. 4) is perhaps a bit surprising. In any case, whether or not there is an added advantage in greater ant activity, our results (Fig. 5) show that *P. icarus* larvae can regulate ant numbers using tentacle display. Larvae with intact tentacles attracted more ants directly after a simulated attack.

In conclusion, larval behaviour in the ant-lycaenid interaction shows a fine-tuned and flexible response to rapidly variable conditions. These responses, together with flexibility in ant tending behaviour, act to produce a dynamically balanced exchange. Where the balance is struck in a particular case depends on variable factors such as larval condition and need for protection, as well as the state of the ant colony. During an interaction, information about this variability is inevitably transmitted between interacting partners, and thus communication becomes an essential element of the relationship.

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