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The larval ant-organs of *Thisbe irenea* (Lepidoptera: Riodinidae) and their effects upon attending ants

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Observations and experiments are presented on the use of larval ant-organs by the riodinid butterfly *Thisbe irenea* with emphasis on their function in myrmecophily. The results indicate that each ant organ plays a distinct role in larval-ant association and that all influence the behaviour of attending ants. Interpretations of the roles that lycaenid larval organs play in ant associations are evaluated and discussed in light of myrmecophilous riodinids. Finally, an 'enticement and binding' process, involving the concerted use of the larval organs, is proposed as the behavioural mechanism that *T. irenea* and other riodinid larvae use to secure the attentions of individual ants for extended periods of time.

KEY WORDS:—Myrmecophilous butterflies – larval ant-organs – Formicidae – Riodinidae – stridulation – amino acid rewards – semiochemicals – evolution and maintenance of ant association.

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INTRODUCTION

Myrmecophilous insects have evolved behaviours and morphological adaptations specifically for living in close association with ants. One of the most

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widespread adaptations involves the development of secretory organs capable of emitting chemicals to modify ant behaviour, or producing ant food (see Wheeler, 1910; Way, 1963; Wilson, 1971, for refs.).

Myrmecophilous relationships of insects can be obligate or facultative. Obligate myrmecophiles require ants to survive. Facultative myrmecophiles do not, but nonetheless gain certain benefits from association with ants, including protection against predators and parasitoids, faster growth rates, higher reproductive success, and aestivation sites (Bartlett, 1961; Banks & Nixon, 1958; Cottrell, 1984; Pierce *et al.*, 1987; Wheeler, 1910; Way, 1963; Wilson, 1971). For both obligate and facultative myrmecophiles the specialized organs mediate the association between the insects and ants.

The butterfly family Lycaenidae (*sensu* Eliot, 1973) contains numerous myrmecophilous species (see summaries in Lamborn, 1915; Farquarson, 1922; Balduf, 1939; Hinton, 1951; Iwase, 1954; Downey, 1961; Clark & Dickson, 1971; Ross, 1966) and all lycaenid larvae bear some specialized ant-organs (Malicky, 1969; Kitching, 1983; Kitching & Luke, 1985; Cottrell, 1984; Henning, 1983; Pierce *et al.*, 1987; DeVries, Harvey & Kitching, 1986). The closely related riodinid butterflies (Riodinidae, *sensu* Eliot, 1973; or Riodininae, *sensu* Ehrlich, 1958) are thought to be composed of mostly myrmecophilous species according to some authors (Hinton, 1951; Callaghan, 1977; Robbins & Aiello, 1982; Pierce, 1987) but not others (DeVries, 1987; D. Harvey personal communication). However, few riodinid larvae have been examined for the presence of ant-organs (see Cottrell, 1984; DeVries *et al.*, 1986; Horvitz, Turnbull & Harvey, 1987).

Considering the diversity of neotropical riodinids (DeVries, 1987), it is surprising that only one species, *Anatole rossi*, has been studied with respect to the role larval ant-organs play in the ant association (Ross, 1964, 1966). The purpose of this study is to describe how the larval ant-organs of another riodinid butterfly, *Thisbe irenea* (Stoll, 1780), influence ant behaviour and what role each organ plays in the larval-ant association. Secondly, a general behavioural model is presented that describes how some riodinid larvae use the ant organs to sustain prolonged attentions of ants.

MATERIAL AND METHODS

I studied the mutualistic association between the riodinid *Thisbe irenea* and its attendant ants from September 1985 to August 1986 on Barro Colorado Island (=BCI), Panama (see Croat, 1978; Leigh, Rand & Windsor, 1982, for site description), where larvae of *T. irenea* are monophagous on leaves of sapling-sized *Croton billbergianus* (Euphorbiaceae) (Robbins & Aiello, 1982; DeVries, 1987). Each leaf possesses a pair of extra-floral nectaries that are tended by ants. First through mid-fourth instars of *T. irenea* rest on the undersides of leaves near or on extra-floral nectaries, and feed intermittently throughout the day. Late fourth to fifth instars rest by day inside dead leaf matter webbed to the host plant or in the leaf litter at the base of the plant, and feed at night. All instars feed on extra-floral nectar in addition to leaf tissue (DeVries & Baker, unpublished). On BCI, both *T. irenea* larvae and the extra-floral nectaries of *C. billbergianus* are tended primarily by *Ectatomma ruidum* (Ponerinae), but if the host plant grows outside of *E. ruidum* territories, larvae are tended occasionally by other ponerine, formicine,

or myrmicine ant species. Although ants clearly protect larvae from predators, *T. irenea* larvae can be reared to adulthood without ants (DeVries, 1987).

Observations of larval organs and ant responses to them were conducted in the field and in the laboratory using naturally occurring plants and ant colonies as well as potted hostplants and captive ant colonies. Laboratory manipulations were performed on larvae grown on potted hostplants with bridges into ant colonies, or on larvae kept in glass Petri dishes where food and ants could be introducted as needed.

Samples of *T. irenea* tentacle nectary organ (TNO) secretion and extra-floral nectar of *C. billbergianus* were collected with 5 μl capillary tubes and spotted on No. 10 Wattman Chromatography paper. Analyses of sugars and amino acids were done by I. Baker, Department of Botany, University of California, Berkeley, using the methods described in Baker & Baker (1976).

Samples of all larval instars were preserved in alcohol. Some were critical point dried, sputter coated with gold/palladium, and then the vibratory papillae and pore cupola organs were examined and/or photographed using SEM facilities at the Department of Zoology, University of Texas, the British Museum (Natural History), and the American Museum of Natural History.

RESULTS

The general morphology and development of larval *T. irenea* and their ant-organs is similar to those described for its close relative *Lemonias caliginea* (in Ross, 1964, as *Anatole rossi*). A detailed account on development and ultrastructural morphology of the myrmecophilous organs of *T. irenea* will be presented elsewhere (Harvey & DeVries, unpublished). In this paper I separate the more generally distributed pore cupolas found on all instars from what I term 'primary ant-organs', the vibratory papillae, TNOs (tentacle nectary organs) and ATOs (anterior tentacle organs), found only on third and later instar larvae (Fig. 1). The terminology for riodinid and lycaenid ant-organs follows Cottrell (1984).

PORE CUPOLA ORGANS

All instars of *T. irenea* larvae have perforated cupola organs (PCOs) scattered on the epidermis that are similar to those described for *L. caliginea* (Ross, 1964), other riodinid larvae (Harvey, 1987), and many lycaenids (Malicky, 1970; Kitching, 1983; Kitching & Luke, 1985; DeVries *et al.*, 1986). The number of PCOs in *T. irenea* increases with each larval instar. Although the role of PCOs in

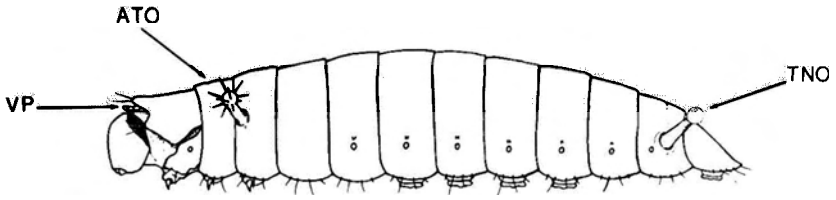

Figure 1. Schematic drawing of fifth instar *Thisbe irenea* larva showing the locations of the primary ant-organs. VP = vibratory papillae; ATO = everted anterior rentacle organ; TNO = everted tentacle nectary organ with a drop of secretion at tip. The pore cupola organs which are scattered across the epidermis are not illustrated (see text).

riodinids is unknown, in some lycaenids they are important for ant associations because they secrete amino acids or adoption substances (Malicky, 1970; Pierce, 1983). If *T. irenea* PCOs play a role in ant association they are probably most important during the first and second instars. However, I never observed ants paying particular attention to the PCOs of *T. irenea* in any instar.

VIBRATORY PAPILLAE
(Figs 1, 3A)

A pair of sclerotized, anteriorly directed vibratory papillae are found on the antero-dorsal edge of the prothorax (segment T–1) and are similar in overall appearance to those described for *L. caliginea* by Ross (1964, 1966). These rod-like structures, which vibrate dorsolaterally and strike the epicranium, are found in a number of riodinid genera. No structures comparable to the vibratory papillae are known from any lycaenid species.

Beat frequencies

Although I was unable to measure the number of times the vibratory papillae struck the head capsule, based on over 100 observations in the field and Petri dishes it was clear the vibratory papillae beat fastest when larvae are stressed (i.e. prodded or gently pinched by the observer), during initial contact with ants after being in isolation for some time, and when larvae are moving to and from a resting area or feeding spot. This was later confirmed by use of sensitive microphones (see below). I estimate that vibratory papillae in a resting larva beat about two times per second, but when the larva is stressed, they beat upwards of 10–15 times per second. When segment T–1 was antennated continually by ants, when larvae were maintained in the absence of ants, or immediately preceding a moult, the vibratory papillae remained motionless.

Sound production

When the vibratory papillae beat frequency is highest, the headcapsule is oscillated rapidly in and out and swung from side to side, resembling the stridulatory behaviour seen in cerambycid beetles. It is significant that ants pay most attention to the head of *T. irenea* larvae when head oscillation rate and the vibratory papillae beat frequencies are highest. I have observed identical behaviour between ants and larvae from the genera *Synargis, Nymphidium, Calospila, Juditha,* and other undetermined myrmecophilous riodinids. Ross (1966) noted that ants will investigate the vibratory papillae of *L. caliginea* larvae and suggested that they may convey vibrations to attract ants. However, no attempt has been made to explain how the vibratory papillae might function in sound production.

Examination of the vibratory papillae and epicranium with the SEM revealed a simple rasp-and file stridulatory system that appears to function as follows. The shaft of each vibratory papilla is densely ringed with concentric grooves and looks like a '*güiro*', the Latin American percussion instrument made of a long, hollow gourd with concentric grooves cut into it. The surface of the epicranium where the vibratory papillae strike bears a scattering of sharp granulations. The stridulatory

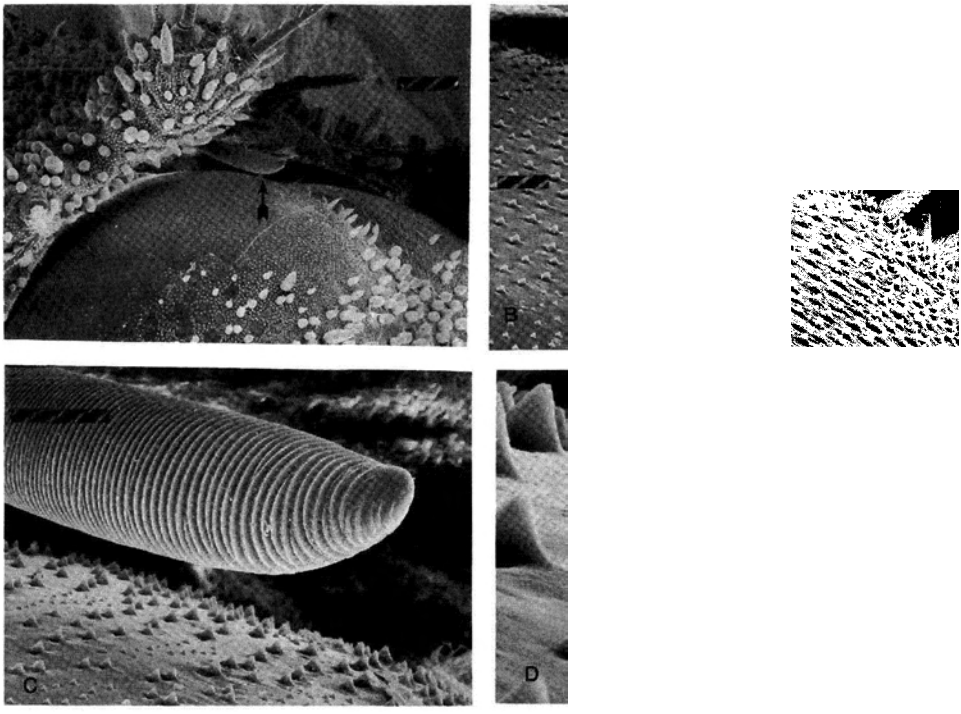

Figure 2. The sound production organs of fifth instar *Thisbe irenea* larva: vibratory papillae (= VP) and granulations on epicranium. A, general view of anterior edge of segment T–1 showing a VP (arrow) and the surface of the epicranium where the VP strikes. Scale bar = 200 microns. B, enlarged view of epicranium and VP. Note the change in density and shape of the epicranial granulations between areas inside and outside of where the VP strikes. The white line is a strand of larval silk. Scale bar = 40 microns. C, detail of the VP showing the annulations on its shaft and the epicranial granulations. Sound production occurs when the head of the larva is oscillated and the epicranial granulations are dragged across the annulations of the VP. Scale bar = 40 microns. D, details showing two sizes of epicranial granulations. Note that both types are directed anteriorly. Scale bar = 4 microns.

mechanism functions when the headcapsule is oscillated back and forth and the grooves of the vibratory papillae are brought into contact with epicranial granulations (Fig. 2). Variation in sound production results with different rates of head oscillation or beat frequency of the vibratory papillae. Recently I have demonstrated that sound is produced only when the action of the vibratory papillae and head oscillation act together and that the sound is substrate borne. An acoustic analysis of the sound and the response of attending ants will appear elsewhere (DeVries, unpublished).

TENTACLE NECTARY ORGANS
(Figs 1, 3)

A pair of eversible tentacle nectary organs (TNOs) are found on segment A–8 which, when viewed with a dissecting microscope, are similar in external morphology to those described for *L. caliginea* (Ross, 1964) and those found in *Synargis, Juditha,* and *Nymphidium* (personal observation). When everted each TNO looks like a finger of a transparent glove and the tip yields a drop of clear

TABLE 1. ATO and TNO eversion frequencies of *Thisbe irenea* during different larval activties and resting positions

	Rest *vs.* Active		
	ATO	TNO	Total
Resting	20	70	90
Active	81	120	201
Total	101	190	291
$g = 9.374$, d.f. = 1, $P < 0.005$			

	Stem *vs.* Nectary		
	ATO	TNO	Total
Stem	8	11	19
Nectary	12	68	80
Total	20	79	80
$g = 6.13$, d.f. = 1, $P < 0.02$			

fluid that is eagerly imbibed by attending ants. The fluid is most likely produced by the large masses of yellowish glandular tissue located within the body wall at the base of the TNO (Ross, 1964, personal observation). If the drop is not imbibed by an ant, it is withdrawn into the body again when the TNO is retracted. The TNOs are analogous, but not homologous, to the single-pore dorsal nectary organ (DNO) found on segment A–7 in lycaenids.

TNO eversion rates

The TNOs of *T. irenea* may be fully everted simultaneously, singly, or partially everted, and the frequency of eversion varies with larval behaviour and number of attending ants. The TNOs remain fully functional up until pupation, including periods during premoult. Except during the initial contact with ants when they remain everted to 'tempt' an attending ant, the TNOs are usually everted only when solicited by ants vigorously antennating the dorsum of segment A–8. In larvae isolated in Petri dishes the highest TNO eversions rates occurred when ants were first introduced into the container, and coincided with the highest vibratory papillae activity. During this phase, both TNOs are usually everted and retracted in rapid succession, although this varies with how fast an ant, or ants can imbibe the fluid reward. About 15 min after the initial TNO eversions the frequency of eversion diminishes, whereupon the ant must solicit TNO eversion by antennating the dorsum of segment A–8. Several hours after initial contact, the attending ant must antennate much more vigorously to elicit eversion of the TNOs. This reluctance to evert the TNOs is typical of captive larvae kept in association with ants for some time or those observed in the field which have had constant ant attendance. In larvae with constant ant attentions the TNO eversion frequency is highest when larvae are moving from one place to another or when feeding on leaf tissue. While at rest eversion frequency is highest while larvae are positioned on or near an extra-floral nectary of the host plant, where they can imbibe nectar (Table 1).

TABLE 2. Amino acid concentrations in TNO secretions of *Thisbe irenea* larvae and *Croton billbergianus* extrafloral nectar. Amino acids are ranked whereby a rank of 1 is the highest concentration. The symbol (?) signifies trace concentrations unable to be measured. Analytic methods are those of Baker & Baker (1976). The total amino acid concentrations are given in micro-moles per microlitre

	Larval number and rank of amino acid					
	No. 1	No. 2	No. 3	No. 4	No. 5	EFN
Alanine	16	15	15	14	18	?
Arginine	4	4	4	5	4	6
Cysteine	7	6	9	9	7	2
Glutamine	8	1	1	1	2	?
Glutamate	18	18	18	16	17	12
Glycine	1	2	2	3	1	13
Histidine	14	9	5	12	14	7
Isoleucine	6	12	8	10	10	9
Leucine	5	3	3	2	6	8
Lysine	9	13	16	0	5	5
Methionine	16	14	17	15	18	14
Phenylalanine	15	7	7	6	9	1
Proline	2	10	14	7	3	11
Serine	10	16	12	4	12	?
Threonine	12	17	13	17	15	?
Tryptophan	11	5	10	8	13	4
Tyrosine	13	8	6	11	11	3
Valine	3	11	11	13	8	10
Concentration	9.4	9.4	12.5	12.5	9.5	1.2

TNO secretion

In fifth instar larvae each drop of TNO fluid available to an ant ranges in volume from 1–3 microlitres ($N = 10$, mean = 1.9, S.D. = 0.88). The secretions are qualitatively very different from the extra-floral nectar of *C. billbergianus* in both the ranks and total concentrations of amino acids and sugars (Table 2). TNO secretion contains almost no sugar (< 0.5%), but is very rich in total amino acids (means of 10.6 μM μl^{-1}: DeVries & Baker, unpublished). The concentrations and ranks of amino acids varied between larvae and probably reflect the physiological state of each larva within its moult cycle. On the other hand, the extra-floral nectar is rich in sugars (33% w/v), but comparatively poor in total amino acids (1.2 μM μl^{-1}), and did not vary between different samples (Baker, personal communication).

In the field and in the laboratory when small *C. billbergianus* plants had a single third or later instar larva plus one or two ants on them, I found that ants tended the larvae, not the extra-floral nectaries. In both captive ($N = 5$) and in wild populations ($N = 3$), marked individual *E. ruidum* ants stayed in close physical contact with individual larvae for 4–10 days and never moved further away than a few centimetres. Rather than leave larvae, these ants passed TNO secretion accumulated in the mandibles to nestmates returning to the colony. One reason why ants seem to prefer larvae over extra-floral nectaries is probably that TNO secretion has higher concentrations of amino acids (Table 2), and in a very real

sense is the currency with which larvae pay attending ants for protection against predators. When the TNOs of fifth instar larvae ($N=5$) were rendered non-functional by applying a drop of nail polish to cover them, ants initially touched the larvae, but soon totally ignored them.

ANTERIOR TENTACLE ORGANS
(Figs 1, 3)

A pair of eversible anterior tentacle organs (ATOs) are found on segment T–3 that are similar in ontogeny and morphology to those of *L. caliginea* (Ross, 1964) and to those found in *Synargis, Juditha* and *Nymphidium* (personal observation). Like the tentacle organs found on segment A–8 of lycaenids, the ATOs of riodinids bear terminal setae communicating via ducts with glandular tissue (Ross, 1964; Malicky, 1970; Cottrell, 1984), and the ATOs (and TOs) are retracted into the body when not in use. Reaching functional development with the third instar, the ATOs of *T. irenea* are used throughout remaining larval life except during periods immediately preceding a moult or when larvae are isolated from ants.

Eversion rates

As in the TNOs, the ATOs of *T. irenea* may evert singly or in pairs, one may evert more frequently than the other, and the frequency of eversion also varies depending on larval activity (Table 1). Eversion is more frequent when larvae are moving or feeding than when they are resting. When at rest, ATO eversion is more frequent when larvae are near nectaries than when on a stem. The ATOs evert only when ants are in bodily contact with larvae, and after eversion they are immediately retracted. They are never everted when ants antennated the orifices on segment T–3. ATO eversion appears to be triggered in some unknown way by ants antennating the area near the TNO orifices, feeding at the TNOs, or standing on the dorsum of a larva. On average an individual ATO remains everted less than 0.5 s, but may rarely remain everted for several seconds (see below). While the ATOs were everted, and for a few moments after they had been retracted, the larvae remained motionless while attending ants vigorously antennated the area immediately surrounding the ATO orifice. However, unlike reports on lycaenid species (Murray, 1939; Clark & Dickson, 1956; Ross, 1966), ants were never observed contacting averted ATOs in *T. irenea*. Lastly, ATO eversion never occurred in the absence of ants, when larvae were stressed by the observer, or when larvae were attacked by predators.

Ant reactions to ATOs

In both captive and in wild populations it was dramatically evident that the everted ATOs of *T. irenea* evoke a strong behavioural reaction in attending ants. In captive fourth ($N=10$) and fifth instar ($N=15$) larvae the first ATO eversions occurred between 1 or 2 min after an introduced ant made physical contact with a larva and was feeding at the TNOs. The initial 1–8 eversions caused ants to momentarily slow down or stop all movement; after a brief pause of 1–2 s, they resumed antennating and feeding at the TNO. The subsequent 8–15 eversions

caused the ant to stop all feeding activity, open its mandibles, and position each antenna into an elbowed, 90° angle. After a pause of a few seconds in this position, ants preened their antennae by drawing them individually through the pectinated spur on the fore tibia, then resumed soliciting food at the TNOs. After 15 or more ATO eversions the ants reacted in a completely sterotyped manner: the mandibles snapped open while the antennae moved into the 90° angle position, the abdomen curled slightly beneath the body, and they literally jumped toward the everted organ. In both captive and wild populations individual ants that are not actively tending the larva do not react to ATO eversion until they too have been sensitized by numerous ATO eversions.

After reacting to the ATO eversion, ants were extremely wary of any nearby movement and when given the oportunity, lunged at and attempted to bite and/or sting any small object moving close by (for example a splinter of wood, a piece of string, or other insects). If no attack stimulus was provided, the ants soon resumed a normal body posture, groomed their antennae, and returned to soliciting TNO eversion. Larvae remained motionless during and after ATO eversion, and ants did not attempt to bite or sting the larvae during this time.

ATOs as ant attractants

To test if ATO eversion in *T. irenea* attracted ants to the TNOs, the ATO of three fifth instar larvae were sealed by placing a drop of clear nail polish over the orifices on segment T–3. In three control larvae drops of nail polish were placed on segment T–3 near the orifices, but not so as to inhibit the function of the ATOs. Individual larvae were placed in separate Petri dishes with pieces of host plant for 1 h, then two *E. ruidum* ants were introduced into the container. After 1 h I noted the interactions between ants and larvae at 10 min intervals during an 8.3 h observation period. Ant behaviour was categorized as: *Ignored,* when ants were not touching the larva; *Touching,* when an ant was touching a larva in any way but not feeding; *Feeding,* when an ant was either soliciting or feeding at the TNOs. Larvae unable to use the ATOs had less physical contact with ants than those with functional ATOs, showing that ATOs may influence ant-larva association, and that although secretion was available, the larvae were unable to maintain the constant attentions of an ant (Table 3).

SUMMARY AND CONCLUSIONS

The primary ant-organs (Fig. 1) in *T. irenea* (vibratory papillae, tentacle nectary organs, and anterior tentacle organs) are similar in ontogeny and morphology to those described for *L. caliginea* by Ross (1964, 1966). These organs function independently, they are used under different conditions and with variable frequency, and are of major importance in maintaining the mutualism between ants and larvae. Each organ provides a distinct stimulus to ants, and all are used repeatedly throughout post third instar larval life. Although pore cupola organs are present on all larval instars, their role in ant association for *T. irenea* remains obscure; ants do not appear to pay particular attention to them, unlike the situation described for lycaenids (Malicky, 1977; Pierce, 1983).

The rod-like vibratory papillae of *T. irenea* are stridulatory organs. Sound is produced when, in conjunction with head oscillation, the ringed grooves on the

TABLE 3. Comparison of ant attentions between six *Thisbe irenea* larvae with non-functional (experimental, $n = 3$) and functional (control, $n = 3$) ATOs. Inspections were done simultaneously every ten minutes during an 8.3 h period

Class	Ignore	Touch	Feeding	Total
Experimental	20	6	8	34
Control	2	13	19	34
Total	22	19	27	68
Ignore X touch:	$g = 16.75$, d.f. = 1, $P < 0.001$			
Ignore X feed:	$g = 20.71$, d.f. = 1, $P < 0.001$			
Touch X feed:	$g = 0.02$, d.f. = 1, $P = 0.887$			
Total log-likelihood:	$g = 24.35$, d.f. = 2, $P < 0.001$			

vibratory papillae are brought into contact with granulations on the epicranium (Fig. 2). These organs are used most vigorously when larvae are kept away from ants or when larvae are stressed. It is my belief that the sound produced by the vibratory papillae is used specifically to attract ants. This idea is strengthened by reports that ants antennate the head region of other riodinid larvae (Bruch, 1926; Ross, 1966, personal observation), and that ants from all subfamilies produce and respond to stridulations or vibrations (Wheeler, 1910; Wilson, 1971; Markl & Holldobler, 1978; Brown, personal communication; Wilson, personal communication). The possession of vibratory papillae and the ability to produce sound in the myrmecophilous riodinid genera *Synargis, Menander, Nymphidium, Calospila, Juditha, Lemonias* and *Audre,* but the absence of both characters in the amyrmecophilous larvae of *Euselasia, Melanis, Charis, Cremna, Emesis, Rhetus, Chorinea, Ancyluris, Mesene* and *Sarota* (DeVries, unpublished) makes it clear that vibratory papillae play an important role in some larval-ant associations. Furthermore, the ability of myrmecophilous riodinids (*Theope, Eurybia*) and lycaenids (several genera) to produce sound even though their larvae do not possess vibratory papillae strongly suggests that sound production is a general adaptation of all myrmecophilous larvae (DeVries, unpublished).

The eversible TNOs of *T. irenea* which are functional in third to fifth instars and produce an amino acid rich liquid (Table 2) that is eagerly imbibed by ants, are clearly important to the maintenance of ant associations. Larvae with TNOs rendered non-functional are ignored by ants, and no amyrmecophilous riodinid species is known to possess TNOs. As in lycaenid larvae (Pierce, 1983), the nutrient rich secretion produced by the TNOs forms the basis of ant-larva mutualisms, and may be considered the currency with which larvae pay for protection against predators. Although first and second instars lack TNOs, they may gain ant protection by remaining in close association with the extra-floral nectaries of the host plant (DeVries, 1987).

The eversion of ATOs by *T. irenea* larvae evokes a marked behavioural response in attending ants and they are important for maintaining ant association (Table 3). There are two interpretations of how lycaenid tentacle organs function. One interpretation suggests that tentacle organs evoke a response in ants by physical or optical means (Murray, 1935; Clark, 1940; Clark & Dickson, 1956; Malicky, 1969). The other broad interpretation suggests that lycaenid TOs disseminate volatile chemicals that evoke responses in attending ants (Clark &

Dickson, 1956; Malicky, 1970; Claassens & Dickson, 1977; Pierce & Mead, 1981; Henning, 1983; Cottrell, 1984; DeVries, 1984). Using chemical extractions Henning (1983) concluded that larval tentacle organs of *Aloeides dentatis* (Lycaenidae) disseminate an alarm pheromone to attract ants, but that tentacle organs in other lycaenid species emit an adoption chemical (see also Pierce, 1983).

The only interpretation of the role ATOs is that of Ross (1966), who concluded that in *L. caliginea* the terminal setae on the ATOs function to drive away predators or persistent ants in a manner suggested for lycaenids (Murray, 1935; Clark, 1940; Clark & Dickson, 1956; Ross, 1966). The hypothesis that the ATOs in *T. irenea* specifically, and riodinids in general, are used as a physical defense to repel predators (Ross, 1966) seems untenable on two grounds: the ATOs do not evert when larvae are molested or attacked by predators, and even if they did evert, the terminal ATO setae are too small to inhibit predatory wasps. Furthermore, the evidence presented here does not support the hypothesis that ATOs attract ants to feed at the TNOs (Ross, 1966). Rather, the ATOs of *T. irenea* appear to produce a volatile chemical that modifies ant behaviour by sensitizing ants through repeated eversions of the ATOs until the ant becomes semi-aggressive. Once an ant reaches a semi-aggressive state, visual stimuli in the form of movement near larvae release an aggressive attack.

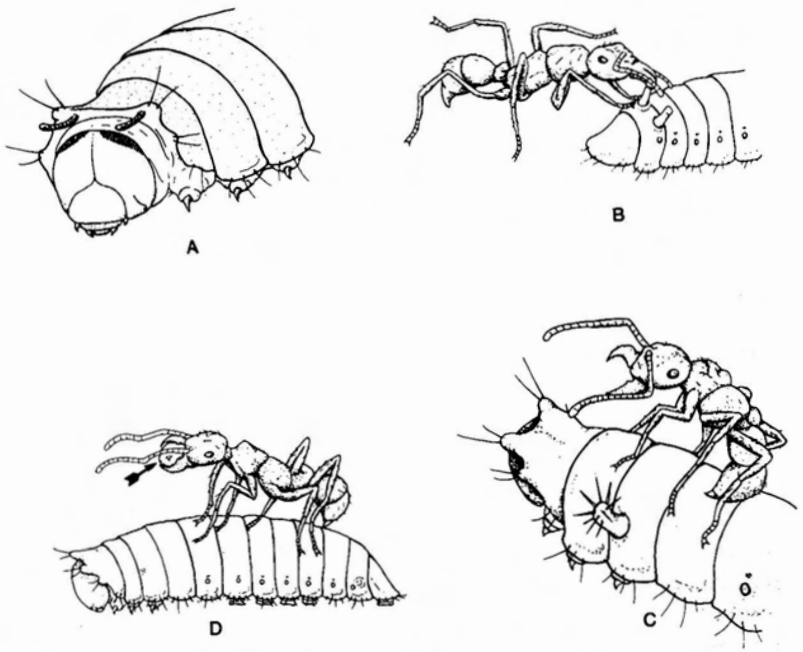

Figure 3. Sequence of 'enticement and binding' whereby a mutualistic ant is manipulated by use of the primary ant-organs. A, larva 'calls' ants by use of vibratory papillae and head oscillation. B, ant imbibing amino acid rich secretion production by the tentacle nectary organs. C, a 'sensitized' ant adopts aggressive posture after exposure to everted anterior tentacle organ. Note stereotyped position of mandibles, antennae, and abdomen. D, the 'bound' ant maintains physical contact with larva for extended periods of time, and rather than leave the larva, the 'bound' ant will pass the accumulation of tentacle nectary organ secretion held in the mandibles (arrow) to a nestmate returning to the colony.

In the alarm pheromone of weaver ants, *Oecophylla longinoda*, a restricted set of trace components act a markers for biting attacks (Bradshaw *et al.*, 1979). Citing this information Cottrell (1984) suggested that an absence of some alarm components from TOs of the *Aloeides dentatis* may explain why Henning (1983) was unable to evoke a biting response in ants exposed to larval extracts. Given the behavioural reactions of ants, I propose that the function of the ATOs of *T. irenea* is specifically to evoke, via chemical means, a defensive attitude in attending ants to prime them for defending the larva against predators moving nearby. As suggested for *A. dentatis* (Cottrell, 1984), the chemicals produced by the ATOs of *T. irenea* will probably be found to lack the chemical markers that induce biting and stinging from ants.

I have found both *Azteca* and ***Monacis bispinosus*** (Dolichoderinae) ants tending a number of riodinid larvae (*Nymphidium, Theope, Juditha, Calospila*) on BCI, yet these ants either kill or ignore *T. irenea* larvae. This may be due to some chemical emitted by larval organs (such as the ATOs) that, while evoking a defensive attitude in ponerine or formicine ants, provokes attack or causes an avoidance response in dolichoderines. An experiment where four *T. irenea* larvae that had been briefly molested by *Azteca* sp. were offered to *E. ruidum* workers in the captive colonies also suggests that chemistry is important in determining which ants associate with larvae: all *T. irenea* larvae were immediately attacked and killed, presumably because they had acquired an *Azteca* odor (DeVries, 1987). These observations allow an alternative explanation for why Ross (1966) found that *Ectatomma* ants attacked and killed larvae of *L. caliginea*. The colony odors from the mutualist ant *Camponotus abdominalis* may have evoked an attack response in *Ectatomma*. Since *Camponotus* and *Ectatomma* both tend *T. irenea* (a close relative of *L. caliginea*), *Ectatomma* may be predators of *L. caliginea* at some sites and mutualists in others, depending upon the density of local ant colonies.

'Enticement and binding' of ants by larvae (Fig. 3)

The benefits of maintaining ant associates during larval life have been demonstrated for both lycaenid and riodinid larvae (Pierce & Mead, 1981; Pierce *et al.*, 1987; DeVries, 1987). Based on the observations presented here, I propose that *T. irenea* larvae are able to sustain ant attentions for up to 10 days through a process I term 'enticement and binding', which describes how a larva attracts ants to it, offers them rewards, and eventually binds individual ants to it through use of the primary ant-organs. 'Enticement and binding' is composed of four major parts (Fig. 3) and is summarized as follows.

1. Larvae call ants by using a stridulatory mechanism composed of the vibratory papillae and head oscillation behaviour (Fig. 3A). The stridulations are produced when larvae are moving or stressed and coincide with times when ant attendance is most necessary.

2. Shortly after initial contact with ants, larvae evert the TNOs and reward them with an amino acid-rich fluid (Fig. 3B). Later ants must solicit the reward by repeated antennation of segment A–8, which then tells the larva that ants are in attendance.

3. Larvae produce a semi-alarmed behavioural state in ants by intermittently exposing them to semiochemicals produced in the ATOs. Repeated ATO eversion

through time causes a sequential alteration of ant behaviour until a strong stereotyped reaction to every ATO eversion is produced in the attending ant (Fig. 3C), thus maintaining individual ants in an alerted state.

4. Individual ants are 'bound' to a larva through continued use of all the primary ant-organs, and thus larvae maintain their ant guards in close physical contact for extended periods of time (Fig. 3D).

The general tendencies of ants to defend food resources (Carroll & Janzen, 1973; Beattie, 1985), forage in specific areas within territories (Rosengren, 1971; Holldobler, 1976; Fowler & Robbins, 1980; Kugler, 1984), the fidelity of individuals to a specific resource (Fowler, 1983), and to show investigatory behaviour in response to the stridulations of nestmates (Wheeler, 1910; Wilson, 1971; Markl & Holldobler, 1978) were likely to have been important behavioural elements in the evolution of ant-larval mutualisms. Furthermore, the responses of ants to the use of *T. irenea* larval organs indicate that their evolution was shaped directly by ants, and the protective benefits of maintaining a constant cadre of ants should select for and refine the concerted use of the larval ant-organs and the sequence of 'enticement & binding'.

The findings of Ross (1966) that individual *Camponotus abdominalis* stay with *L. caliginea* larvae for several days suggest that these ants were 'bound' to larvae. The responses to larval ant-organs described here for *T. irenea* have also been observed in ants tending larvae of *Synargis, Juditha,* and *Nymphidium* (personal observation). Therefore I propose that enticement and binding generally describes how many riodinid species, and perhaps lycaenids as well, maintain their larval ant associates.

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