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Is the larval and imaginal signalling of Lycaenidae and other Lepidoptera related to communication with ants?

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The larval instars, pupae and adults of ant-associated lycaenid butterflies have to integrate into the ants' communication system in order to appease the normal aggressive behaviour of the ants and hence to profit from protection from predators. The vibration signalling of caterpillars, pupae and adults (imagines) was studied and its impact on the appeasement of ant aggressiveness after the emergence of the butterfly from the pupal skin was tested. As the vibration signalling in the larval instars and the emerging butterfly is only found in lycaenid species, which are at the same time the species with ant-attendant relationships in their life-cycle, the vibration might play a role as manner of communication. During emergence, the communication only works well in the Polyommatini, while other lycaenids evoke aggressive behaviour in the ants and get killed easily. In case of adults a pheromonal substance might also be involved.

Speelt het larvale en imaginale vibratie signaal van Lycaenidae en andere vlinders een rol bij de communicatie met mieren? – De larvale tussenstadia, poppen en volwassen exemplaren van met mieren geassocieerde blauwtjes (vlinders) zijn genoodzaakt zich te integreren in het communicatie systeem van de mieren om het normale agressieve gedrag van deze dieren te kalmeren. Het doel is te profiteren van de bescherming die mieren bieden tegen predatoren. De vraag is of het vibreren van de blauwtjes daarin een rol speelt. Daartoe werd het vibratie signaal van rupsen, poppen en imago's van verscheidene vlindersoorten bestudeerd en werd bekeken in hoeverre het signaal de agressiviteit van mieren deed afnemen nadat de vlinders uit de pophuid gekropen waren. Aangezien het afgeven van een vibratie signaal door de larvale tussenstadia en de uitkomende vlinder uitsluitend bij Lycaenidae-soorten (die tijdens hun levenscyclus regelmatig door mieren bezocht worden) werd vastgesteld, wordt geconludeerd dat het vibreren een belangrijke rol kan spelen bij de communicatie tussen vlinder en mieren. Tijdens het uitkomen van de pop bleek de communicatie uitsluitend goed te werken bij Polyommatini-soorten, terwijl andere Lycaenidae-soorten agressief gedrag bij de mieren ontlokten en direct werden gedood. Sommige vlinders (imago's) produceren ook een feromoon om de mieren te kalmeren.

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INTRODUCTION

For more than 200 years we know that the pupae of lycaenids produce sounds (Kleemann 1774). Prell (1913) detected the sound organs in the intersegmental split between the 5th and 6th abdominal segment. On the 6th segment there is a dentated plate and on the 5th segment a vibrating plate is present. The plates produce a sound when rubbed together by muscular contractions. For more than 40 lycaenid species it has been shown that this kind of stridulation is a normal behaviour of pupae (Elfferich 1988, 1989; Elfferich & Jutzeler 1988). Downey (1966, 1967) tested a number of nearctic species and Downey & Allyn (1973) analyzed the sound organs by SEM. In his study of the Riodinidae, DeVries (1990) discovered sound producing organs on caterpillars: he tested lycaenid caterpillars and found vibration signals too (DeVries 1991a, b). Often larval instars of lycaenid species have relationships with ants. These relationships can be quite weak, like in hairstreaks (Theclinae) which do not form stable relationships with ants. Most lycaenids, for example the *Plebejus* and *Polyommatus* species, are steadily myrmecophilous: most mature larvae live ant-associated (Fiedler 1988; Fiedler 1991; Schurian & Fiedler 1991). The most intense relationship is found in *Maculinea*-species, who spend nine months of their caterpillar life as parasites in *Myrmica* nests (Elfferich 1963; Thomas & Elmes 1987). As ants live in high numbers in organized colonies with highly developed communication systems (Hölldobler & Wilson 1990) and are potential caterpillar-predators, the main problem for the caterpillars is to integrate into the ant communication system. The close relationship with the ants gives rise to the idea that, next to adaptations to prevent predation, some kind of communication takes place. It is not unlikely that the vibration of caterpillars, pupae and possibly adult butterflies is a way to communicate with ants.

In this study, many caterpillars, pupae and imagines were tested for vibrational signalling. Furthermore, the importance of vibrations of adults directly after emergence from the pupal skin in the presence of ants was studied.

MATERIAL AND METHODS

Collection of test species

Most of the caterpillars, pupae and butterflies used for the experiments have been bred from eggs laid by females in captivity. Most females were captured in The Netherlands or other countries in Western Europe. The eggs of *Polyommatus icarus* were collected in Crete, Greece. Some eggs were kindly given by other entomologists.

Vibration recording

The vibration signals of caterpillars, pupae and adults could only be made audible by means of a contact microphone. They were simultaneously recorded via a record-player-element and by a specially prepared microphone (Telefunken TD-20). The insects were placed on a piece of sigarette-paper placed directly on the microphone membrane. In this way, it was also possible to record the vibrations of very small caterpillars, such as *Maculinea nausithous*. To record the vibrations after emergence of adults and during the wing expansion, a small light T-shaped perspex construction was made. This was placed upside down on the microphone membrane. A light piece of a twig was glued on the top of the vertical leg under a small angle. Most of the just emerged butterflies moved to the highest place to stretch the wings. To test whether the sound during eclosion was produced by the pupal sound organ, the pupal skin was removed from the abdomen of some *Polyommatus icarus*. This was done 45 to 30 minutes before the expected emergence. Furthermore, videorecordings were made by means of a Watec CCD colour-camera (Wat-202) and a JVC S-VHS videorecorder.

Testing the reaction of ants on eclosing adults

Some tests were run to observe the behaviour of the ants towards newly hatched butterflies. These tests were done in plastic boxes connected with plasternests where the ant colonies were kept. These boxes were normally in use as a feeding place for the ant colony. A few days before emergence the pupae were placed into the plastic box. The behaviour of the emerging butterfly and the ants were recorded with a JVC GR-77 videocamcorder.

RESULTS

Caterpillar vibrations

All tested lycaenid caterpillars regardless of their taxonomic group, always produced a

Table 1 Caterpillars tested for vibration (this study).

no vibration	vibration
Pieris napi (L)	*Lycaena helle* (D&S)
Melanargia galathea (L)	*Lycaena phlaeas* (L)
Epirrita dilutata (D&S)	*Heodes tityrus* (Poda)
Gymnoscelis rufifasciata (Haw)*	*Quercusia quercus* (L)
Mimas tiliae (L)	*Satyrium ilicis* (Esp)
Adscita statices (L)	*Satyrium w-album* (Esp)
Zygaena lonicerae (Schev)	*Callophrys rubi* (L)
Sideridis albicolon (Hüb)	*Maculinea teleius* (Bergstr)
	Maculinea nausithous (Bergstr)
	Zizeeria knysna (Tr)
	Celastrina argiolus (L)
	Philotes baton (Bergstr)
	Plebejus argus (L)
	Vacciniina optilete (Knoch)
	Lysandra coridon (Poda)
	Polyommatus icarus (Poda)

*The caterpillars of *Gymnoscelis rufifasciata* reacted by drumming with their legs, resulting in a vibration-like signal

humming vibration. However, no vibration was recorded with caterpillars of other Lepidoptera (Table 1). There was no detectable difference between the vibrations produced by myrmecophilous and non-myrmecophilous species. Furthermore, in the audio- as well as in the video-recordings no changes in vibration as a reaction to visiting ants could be found. After physical contact with ants there were no reactions either. The vibrations were also produced by the *Maculinea nausithous* and *M. teleius* larvae in the adoption stage. During the pupation, vibrations are produced too. A sound producing organ was searched for in the caterpillars but could not be found. Only in the pupal instar, when the sound organs are developed, the vibrations result in a stridulation signal that is audible.

Butterfly vibrations during and after emergence from the pupal skin

During eclosion, all lycaenid butterflies gave strong vibrational signals whereas the non-lycaenid species never produced vibrations (Table 2). This was remarkable because sounds were recorded at the moment the butterfly's abdomen was already separated from the pupa. Some more tests were run with fully developed pupae of *Polyommatus icarus* that were treated in such a way that the pupal skin with the sound organs was separated from the abdomen just before eclosion. The newly emerged butterflies also showed vibrations. During the emergence of the butterflies, vibrations were recorded. At the experimental construction it was possible to record vibrations after the emergence during the period when the insect showed hestitating movements to find a place for expanding the wings. After that, the imago stopped giving vibration signals. However, after the expansion of the wings, a slight vibration signal was recorded at the moment the imago squeezes its wings together. Tests with other freshly emerged butterflies of other groups and moths gave comparable results (Table 3). Once being able to fly, no vibrations were detected in any tested butterfly species.

Butterfly vibration and ant behaviour

Polyommatus icarus (Rott) and *Plebejus argus* (L) were not attacked during the emergence of the butterflies from the pupal skin in the nest of *Lasius niger* (L), but *Lycaena phlaeas* (L) and *Satyrium ilicis* (Esp) were killed. In a nest with *Myrmica ruginodis* (Nyl) all four butterfly species were killed. Imagines of *Polyommatus icarus* (Rott), *Plebejus argus* (L) and *Zizeeria knysna* (Tr) could escape without any harm from the colonies of *Lasius niger* (L). After the butterfly had left the pupal skin, the ants follow the footmark of the 'protected' butterfly. On spots where the imago stopped for a moment during the search for a good spot for wing-expansion, after a few seconds some ants gathered. During the expansion and 'drying' of the wings the ants crawled freely over the butterfly. After about one hour, as the imago is ready to fly, the ants started to show aggressive behaviour, but then the butterfly could easily escape by flying away.

Table 2 Imagines of butterflies tested for vibrations during the emergence from the pupal skin (this study).

vibration	no vibration
Lycaena helle (D&S)	*Hamaeris lucina* (L)
Lycaena phlaeas (L)	*Pieris brassicae* (L)
Heodes tityrus (Poda)	*Leptidea sinapis* (L)
Heodes ottomanus (Lef)	*Pieris rapae* (L)
Quercusia quercus (L)	*Vanessa atalanta* (L)
Thecla betulae (L)	*Inachis io* (L)
Satyrium ilicis (Esp)	*Araschnia levana* (L)
Callophrys rubi (L)	*Melitaea didyma* (Esp)
Lampides boeticus (L)	
Zizeeria knysna (Tr)	
Celastrina argiolus (L)	
Philotes baton (Bergstr)	
Plebejus argus (L)	
Vacciniina optilete (Knoch)	
Aricia agestis (Schiff)	
Lysandra coridon (Poda)	
Polyommatus icarus (Poda)	

Table 3 Butterfly species producing slight vibrations after expansion of the wings (this study).

Lycaena phlaeas (L)
Maculinea nausithous (Bergstr)
Polyommatus icarus (Rott)
Pyrgus malvae (L)
Pieris brassicae (L)
Pieris rapae (L)
Vanessa atalanta (L)
Aglais urticae (L)
Araschnia levana (L)
Melitaea didyma (Esp)
Leptidea sinapis (L)
Deilephila elpenor (L)
Melanchra persicariae (L)
Abrostola triplasia (L)
Euclidia glyphica (L)
Callistege mi (Clerck)
Adscita statices (L)
Phragmatobia fuliginosa (L)
Epione repandaria (Hufn)

CONCLUSION AND DISCUSSION

The caterpillars and pupae of all tested lycaenid species are able to produce vibration signals, while the caterpillars of all other Rhopalocera and moth species do not show this behaviour. Although it seems to be a normal lycaenid behaviour, no difference could be found in the frequency and intensity of vibrations in relation to the intensity of myrmecophily. The vibrations can best be seen as a method of general communication between lycaenids and ants, rather than enabling species-specific communication. The fact that *Maculinea* species are not able to establish a fine tuned communication by vibrations with their specific host ants (De Vries et al. 1993), also appears to apply to all lycaenid caterpillars. During the emergence from the pupal skin, again, only the lycaenid species produce vibrations. However, during the last stage of wing expansion, all butterfly species could give a slight vibration during a short period (not to be confused with the warming up vibrations before taking off).

The ant-attendant life style, albeit in various intensities, is among the European Lepidoptera only known from the lycaenids. As the ability to produce vibration signals is also restricted to lycaenids, (no other tested butterfly species showed this behaviour), signalling by vibrations could well be related to communication with ants. The protection through 'speaking foreign languages' is sufficient to protect larval and pupal instars from attacks by ants. They are obviously able to appease the natural aggressiveness of the ants. Once being a butterfly, this way of communication cannot guarantee full understanding anymore. In the presence of certain ant species, only the Polyommatini survived eclosion.

Critical analysis of the sound-recordings of different groups of Lycaenidae during the emergence revealed just a small difference between the Polyommatini and the other groups. Just before the emergence all lycaenids give strong signals but in the Polyommatini, in most cases, the vibrations last somewhat longer, probably because of the slower emergence from the pupal skin. During and after eclosion, the influence of communication by vibration to appease the ants is of minor importance. Based on the behaviour observations, it appears more likely that some lycaenid butterflies produce a pheromonal substance to calm the ants. This pheromone is only successful in some ant species, and is only effective for a short period.

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