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RESEARCH ARTICLE

Eversible tentacle organs in caterpillar–ant communication: do they signal partner quality in polyommatinae lycaenid butterflies?

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Abstract

In mutualistic associations, lycaenid butterfly caterpillars trade nectar secretions against protective services by ants. Eversions of paired abdominal tentacle organs (TO) have been suggested to honestly signal nectar secretion capacity of caterpillars to their ant visitors. Using data from 1561 staged encounters between larvae of nine West Palaearctic Polyommatinae species and worker ants of three species, I show that within experimental series, significantly positive correlations between TO eversion and nectar secretion rate emerged only sporadically (12 of 50 series). In a meta-analysis integrating over all tested species combinations and experimental conditions, the relationship between nectar secretion and TO eversion rate was weak ($r^2 = 6.6\%$), though significantly positive. This association between myrmecophilous behaviours was not stronger in experiments with feeding mature larvae than in prepupal non-feeding larvae; however, the latter delivered distinctly more nectar. Relationships between nectar secretions and TO eversions were independent of the density of ant partners available and did not vary consistently between lycaenid species showing different levels of myrmecophily. When mean values of nectar secretions per experimental series were related to the respective mean TO eversion rates, a clear positive relationship only emerged among intimately ant-associated species. In moderate myrmecophiles average tentacle activity was unrelated to mean nectar delivery. Overall, these experiments yielded only weak support for the reliable-signalling hypothesis. I propose that TO eversions rather serve as complementary dimension of multimodal communication between partners. 'Apparent honesty' may then emerge if caterpillars achieve optimal ant attendance by concomitantly increasing nectar secretion and TO eversion rates.

Keywords Lycaenidae · Ants · Mutualism · Communication · Myrmecophily · Manipulation

Introduction

More than half of the over 5000 extant species in the butterfly family Lycaenidae associate with ants during part of their life cycle (Fiedler 1991; Hölldobler and Kwapich 2022; Pierce and Dankowicz 2022a, b). Interactions between lycaenid butterfly larvae and ants are conveyed by a concert of chemical and acoustic signals (Fiedler et al. 1996; Barbero et al. 2009; Sala et al. 2014; Schönrogge et al. 2017; Casacci et al. 2019; Pierce and Dankowicz 2022a). Two types of epidermal organs are particularly important in mediating caterpillar–ant associations. First, from their dorsal nectar organ situated on the 7th abdominal segment (DNO hereafter) lycaenid larvae may deliver secretion droplets rich in carbohydrates and amino acids (Daniels et al. 2005). Second, paired eversible tentacle organs (TO hereafter; Gnatzy et al. 2017) occur in many, but by no means all myrmecophilous lycaenids (Fig. 1). If present, TO are situated latero-dorsally on the 8th abdominal segment of a lycaenid caterpillar.

Within the Lycaenidae, larval TO are known to occur in the subfamilies Curetinae, Aphnaeinae, and the non-monophyletic Polyommatinae and Theclinae (Kawahara et al. 2023), whereas they appear to be completely lacking in Poritiinae, Miletinae (except the African genus *Aslauga*), and in Lycaeninae (Fiedler 1991). TO are absent in a number of lineages which comprise myrmecophilous species (Fiedler 1991, see phylogenies in Espeland et al. 2018 and Kawahara et al. 2023), including most lycaenids whose larvae parasitize ant colonies (e.g., *Phengaris, Lepidochrysops*: Fiedler 1998). Hence, distribution of TO across taxonomic or ecological groups suggests that they are neither necessary

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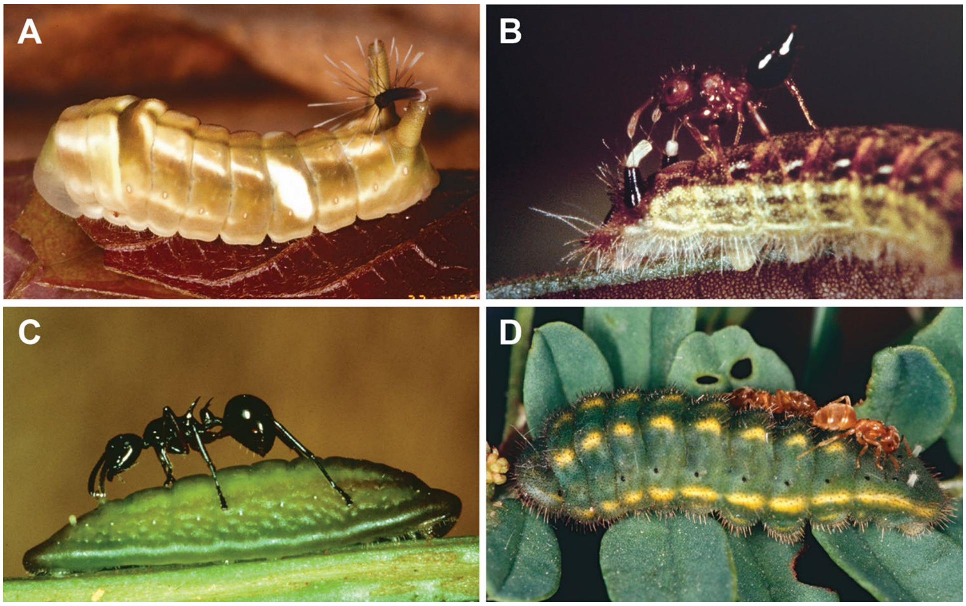

Fig. 1 Diversity of eversible abdominal tentacle organs (TO) in four clades of Lycaenidae butterflies. A: *Curetis bulis* (Westwood, 1852) (Curetinae). B: *Cigaritis lohita* (Horsfield, [1829]) (Aphnaeinae), attended by *Crematogaster dohrni artifex* Mayr, 1879. C: *Eooxylides tharis* (Geyer, [1837]) (Theclinae), attended by a *Polyrhachis* sp. D: *Lysandra bellargus* (Rottemburg, 1775) (Polyommatinae), attended in a laboratory encounter by *Lasius flavus* (Fabricius, 1782). Only species with TO of the type in D (with small and entirely membranous TO) were considered in the present experimental study

for maintaining myrmecophilous associations, nor is their existence invariably linked to myrmecophily. Views about the TO's function remain controversial. Usually, in myrmecophilous lycaenids TO are everted only when a caterpillar is in contact with ants, suggesting that they are indeed involved in communication with ants. On the other hand, excessively large TO occur in non-myrmecophilous Curetinae (Fig. 1A) where they rather serve as defensive organs (DeVries 1984; Fiedler et al. 1995). Unusually large TO also occur in Aphnaeinae caterpillars (Fig. 1B) which are predominately obligate myrmecophiles (Heath 1997; Boyle et al. 2015; Basu and Kunte 2020). In Theclinae and Polyommatinae caterpillars, in contrast, TO are usually small and entirely membranous (Fig. 1C, D). In all nine studied species of Polyommatinae, the TOs are not associated with any sclerotized structures like the rigid base tubes in Aphnaeinae or Curetinae (Fig. 1A, B).

Several hypotheses have been proposed on the significance and function of the TO in caterpillar–ant communication. Earlier authors suggested that TO may serve to attract ants to caterpillars from a distance, or to direct ant visitors towards the DNO. Others supposed that TO are most often used when a caterpillar's secretory capacities are exhausted or help to maintain the integrity of an association when caterpillars move between feeding and resting sites (summarized in Henning 1983). Attendant ants may often, though not invariably, become alerted through TO eversions (Fiedler and Maschwitz 1988; Ballmer and Pratt 1991). The chemical or physical nature, however, of any stimuli involved remains enigmatic. In certain African Aphnaeinae, TO have been suggested to mimic components of ant alarm pheromones (Henning 1983). Yet, recent research on TO ultrastructure in a few Polyommatina species failed to provide evidence for any glandular cells, even though unusual flocculent material and vesicles with unknown content were discovered (Gnatzy et al. 2017).

About 30 years ago, Leimar and Axén (1993) and Axén et al. (1996) proposed that, by alerting ants with their TO, *Polyommatus icarus* larvae signal their ability and alacrity to deliver nutritious nectar droplets to their ant visitors. According to their experiments, larvae of *P. icarus* increased TO eversion rates as well as DNO secretion rates under simulated predator attacks, or else in situations where the attraction of more ant guards should be advantageous. In contrast, activity of both organs levelled off at high ant visitor densities, when a caterpillar should have no need to further invest energy in attracting additional ants. Accordingly, TO eversions could be viewed as reliable 'cost-added signals' (Smith and Harper 1995).

Experiments on temporal and developmental patterns of myrmecophilous behaviours likewise revealed evidence consistent with the reliable-signalling hypothesis. DNO as well as TO tend to be more active at the onset of interactions as compared to long-lasting well-established associations (Burghardt and Fiedler 1996b; in line with an 'enticement and binding' strategy: DeVries 1988). Myrmecophilous behaviours of caterpillars were generally found to be finely tuned in response to the number of ants present and the stability of ant associations (Fiedler and Hummel 1995; Fiedler and Hagemann 1995). However, the activity of TO often varies tremendously between individuals of the same species, between different experimental series with the same caterpillar species, and across closely related species. A better understanding of this variation is thus crucial for evaluating the role which TO might play in lycaenid–ant interactions. Here I test two predictions derived from the reliable-signalling hypothesis, using a range of European Polyommatinae species with ant associations varying from loose to intimate, but excluding obligate and host-specific myrmecophiles. In the latter, host-specific signals may provide a different setting for communication between both parties (Fiedler 2021).

(1) Within species, activity of eversible tentacle organs is correlated positively with nectar droplet delivery. Such relationships hold at all developmental ages and under various conditions. The strength of such a relationship then offers a measure of signal reliability.
(2) Across species, mean nectar secretion and tentacle eversion rates are likewise correlated, with particularly high TO activity observed when caterpillars are most profitable as nectar producers, i.e., in their prepupal phase, or in case of intimate ant associations.

Materials and methods

Butterfly species. Experiments were conducted with nine West Palaearctic lycaenid species all of which maintain facultative ant associations during later stages of larval development (Table 1). Larvae of five test species are moderate myrmecophiles, i.e., less than 50% of larvae found in nature are attended by ants: *Celastrina argiolus*, *Polyommatus amandus*, *P. icarus*, *P. cornelia*, and *Zizeeria knysna*. In contrast, caterpillars of *Lysandra coridon*, *L. bellargus*, *Aricia agestis*, and *Glaucopsyche alexis* have more intimate, though unspecific facultative ant associations and last instar caterpillars are rarely found without attendant ants (e.g., Fiedler 2021 for a review).

Larvae were reared in the laboratory from eggs laid by captive females, except for *L. coridon* which were collected in the field as young instars (L2–L3). Stock for experiments

Table 1 Synopsis of the experimentally tested combinations of lycaenid butterfly larvae and ants, with further specifications

Lycaenid species	Instars tested	Ant species	Ant numbers	Type of association	Duration of experiments (min)	Larval food
Polyommatus icarus (Rottemburg, 1775)	L3, young and mature L4, prepupa	*Lasius flavus, Lasius niger*	1 / 5 / 15	Temporary	15	Flowers of *Lotus corniculatus, Medicago sativa, Melilotus officinalis, Securigera varia*; foliage of *L. corniculatus, M. sativa, S. varia*; semi-synthetic diet
Polyommatus cornelia (Gerhard, 1851)	L4, mature L5, prepupa	*Lasius flavus*	15	Temporary	30	Flowers of *L. corniculatus* before diapause; foliage of *M. sativa* after diapause
Polyommatus amandus (Schneider, 1792)	L3, mature L4, prepupa	*Lasius flavus, Lasius niger*	15	Temporary	15	Foliage of *Vicia cracca, L. corniculatus, M. sativa*
Lysandra coridon (Poda, 1761)	L3, mature L4, prepupa	*Lasius flavus, Lasius niger*	15	Temporary	15	Foliage of *S. varia*
Lysandra bellargus (Rottemburg, 1775)	mature L4	*Lasius flavus*	10	Temporary	15	Foliage of *S. varia*
Aricia agestis ([Denis & Schiffermüller], 1775)	mature L4, prepupa	*Lasius flavus*	1 / 2 / 5 / 10, 5 / 10	Permanent, temporary	30 30 / 15	Foliage of *Geranium molle*
Zizeeria knysna (Trimen, 1862)	L3, mature L4, prepupa	*Lasius flavus, Myrmica rubra*	5 / 15	Temporary	15	Foliage of *M. sativa,* semi-synthetic diet
Glaucopsyche alexis (Poda, 1761)	mature L4	*Lasius flavus*	10	Temporary	15	Flowers of *M. sativa*
Celastrina argiolus (Linnaeus, 1758)	mature L4, prepupa	*Lasius flavus*	15	Temporary	15	Flowers of *M. sativa,* unripe fruits of *Cornus sanguinea*

originated from northern Bavaria, except for *P. cornelia* (southern Turkey: Fiedler et al. 1994) and *Z. knysna* (Canary Islands: Fiedler and Hagemann 1995; Daniels 2004). Experimental larvae were reared singly or in small groups in transparent plastic boxes on freshly cut hostplant material or semi-synthetic diet. Food was offered in ample supply and exchanged daily or every second day, as needed. Larvae were kept under 22.5 °C and 18:6 h L:D cycle. Larval nutrition may strongly affect the expression of myrmecophily (Burghardt and Fiedler 1996a). Rearing the more polyphagous species (*P. icarus, C. argiolus, Z. knysna*) on a range of diets of different quality thus provided an opportunity to experimentally manipulate secretory capacity and thereby amplify variation in the delivery of DNO secretions. Combinations of butterfly species, ant treatments, and food resources offered are summarized in Table 1.

Myrmecophilous behaviours of lycaenid larvae often change with age and development. Non-feeding prepupal larvae typically deliver distinctly more DNO secretions than still feeding, mature final instars of the same size (Fiedler and Hummel 1995; Burghardt & Fiedler 1996b). Therefore, experimental series of the different developmental age classes within the final instar (viz. young, mature, and non-feeding prepupal larvae) were analysed separately.

Ant species. I used two *Lasius* and one *Myrmica* species for experiments. The majority of trials ($n=969$) were conducted with *L. flavus* (Fabricius, 1782). *L. flavus* ants rarely tend lycaenid larvae in nature due to their subterranean life habits (Seifert 2018). Yet, they provide excellent test organisms for quantitative studies of lycaenid–ant interactions because of their strongly developed trophobiotic behaviour (diet almost exclusively consisting of root aphid honeydew: Pontin 1978). *L. niger* (Linnaeus, 1758) ($n=562$ tests) is an omnivorous ant species engaged in trophobiotic mutualisms with a variety of honeydew producers and is amongst the most prevalent ant species to attend lycaenid larvae in Central Europe (Fiedler 2021). *M. rubra* (Linnaeus, 1758) is another omnivorous ant species (Seifert 2018) that has been recorded as tending a multitude of European lycaenid species in nature.

Experiments. Myrmecophilous behaviours of caterpillars were recorded during staged encounters with a fixed number of ants as potential mutualists. I here report on results obtained in 1561 experimental encounters with individual larvae, distributed across 50 experimental series. The worker ants required (usually 15, but fewer in some experimental series, see Table 1) were carefully taken with a brush from laboratory colonies of the respective ant species and transferred into a test arena (a plastic box, size 10×10 cm^2, the bottom lined with plaster of Paris and moistened for each trial). For experiments only foraging ants on their way to a food source were used. The same set of ants was used for a maximum of three successive trials, segregated by pauses of at least 10 min. Ants were allowed to accommodate to the test arena for 5 min. Then a single test caterpillar was placed in the middle of the arena. Beginning with the first contact between the caterpillar and ants, which usually occurred within seconds after introducing the larva, all secretion droplets delivered from the DNO and all TO eversions were counted over the test period (15 min, but 30 min with *P. cornelia* and *A. agestis*). Under these experimental conditions, stable associations between a test caterpillar and ants (Fig. 2) usually established within seconds and persisted for many hours (K. Fiedler, unpublished observations).

Observations were done under a stereo-microscope at tenfold magnification to ensure that no nectar secretion act went unnoticed. Simultaneous eversion of both TO was counted as one single event. During experimental encounters with ants, caterpillars had no access to food. Experiments were conducted during daytime under ambient light and temperature conditions in the lab (22–27 °C). The bio-assay procedure was described in detail in Burghardt & Fiedler (1996b).

Modifications of this general procedure occurred with regard to three parameters. First, the number of potential ant visitors was varied with three butterfly species (*P. icarus, A. agestis, Z. knysna*) to investigate pos1995sible effects the density of ants may have on myrmecophilous behaviours (Leimar & Axén 1993; Fiedler & Hagemann 1995; Fiedler & Hummel). Second, most larvae were in contact with ants only during the staged encounters. However, in *A. agestis* tests were also conducted with larvae which were permanently kept together with ants from the beginning of their third instar until pupation (Fiedler & Hummel 1995). Third, most experiments were conducted with last instar larvae, but some series of experiments also involved pre-final instars.

Data analysis. From each staged encounter, two data points were included in the analyses: the number of nectar

Fig. 2 Representative outcome of a staged encounter in a test arena between a mature fourth instar caterpillar of *Polyommatus icarus* and *Lasius flavus* worker ants. Six of the 15 ants in the arena surround the dorsal nectar organ on the 7th abdominal segment, while another one palpates the prothorax

secretion droplets delivered over the entire test interval; and the frequency of TO eversions. Relationships between DNO and TO activity per individual, within each experimental series, were then expressed as Pearson correlation coefficients r, calculated from log-transformed raw data ($\log(x+1)$) to alleviate the influence of occasional high outlier counts. These r values were then converted into Fisher's z statistics as a measure of effect size. From the effect sizes of the 50 experimental series, I calculated a grand mean using the meta-analysis function in JASP 0.18.3.0 (JASP Team 2024). Effect sizes were also compared between groups of experimental series to assess whether observed differences in the contingency between DNO and TO activity were larger than expected by chance. For the second analysis (i.e., across species), I calculated the mean TO eversion and nectar delivery frequency (log-transformed) for each experimental series and checked these means for correlations. Graphical and statistical analyses were done using the packages PAST 4.16c (Hammer et al. 2001) and JAMOVI 2.5.3 (retrieved from https://www.jamovi.org/download.html).

Results

Relationship between DNO and TO activity within experimental series

Among the 50 experimental series, 36 correlation coefficients between TO eversions and nectar droplets delivered (72%) were positive in sign, and 14 were negative (Fig. 3, Table 2). Robust positive correlations (i.e., confidence intervals of Fisher's z not overlapping zero) were seen in 14 experimental series, namely with *Polyommatus icarus* (8 series), *P. cornelia* (1), *Aricia agestis* (3), and *Zizeeria knysna* (2); five of them rested on large sample sizes (> 100 trials). Aggregating all 50 series in a meta-analysis, a weak, though highly significant positive effect could be confirmed (grand total; Fisher's $z=0.263\pm0.032$ SE, $z=7.693$, $p<0.001$). After back-transformation this corresponds to a grand total correlation coefficient between nectar secretions and TO eversions, across all available experiments, of $r=0.257$ ($r^2=0.066$).

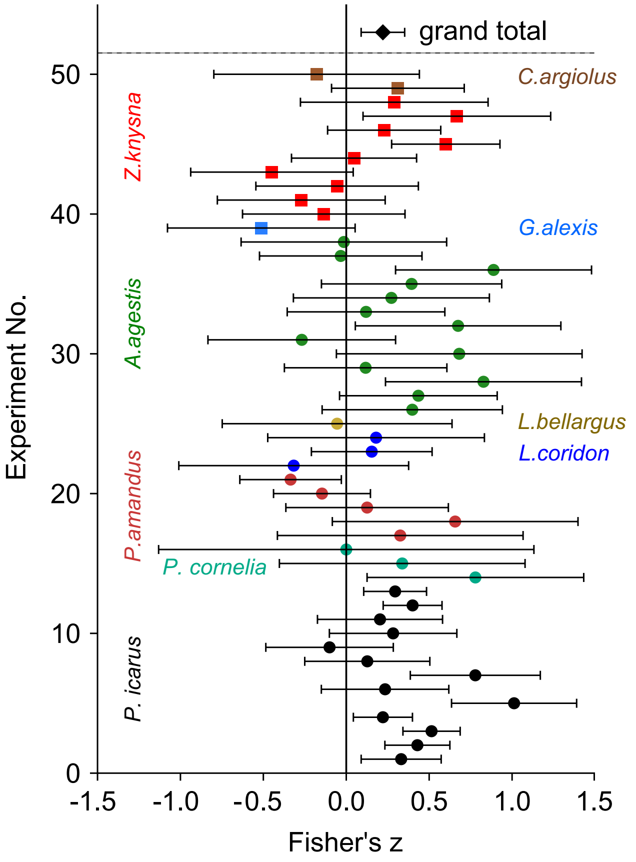

Fig. 3 Forest plot of effect sizes (Fisher's z) derived from Pearson correlation coefficients between nectar secretion and tentacle eversion rates, observed within 50 series of experiments. "grand total" denotes the grand mean from a meta-analysis across all series. Filled dots: species of subtribe Polyommatina sensu Talavera et al. (2022); filled squares: species outside Polyommatina

Effect sizes for DNO-TO correlations did not differ significantly between experiments with moderately versus intimately ant-attended species ($t_{48}=0.152$, $p>0.8$), nor between experiments with high versus low ant densities ($t_{48}=1.44$, $p=0.156$). Also, between the age classes of caterpillars (pre-final instars, actively feeding last instar caterpillars, and non-feeding prepupal caterpillars) there was no significant difference in the tightness of DNO-TO correlations (ANOVA: $F_{2;47}=1.20$, $p=0.31$), even though prepupal caterpillars invariably delivered substantially more nectar droplets than mature, still feeding ones (Wilcoxon test on means from 19 paired series: $z=3.7828$, $p<0.0001$). In contrast, average TO activity did not significantly differ between feeding and prepupal final instar larvae in these same subsets of experimental series (Wilcoxon test: $z=1.6092$, $p=0.0951$). The identity of the ant species used in experiments had no effect on the strength of DNO-TO correlations (ANOVA: $F_{2;47}=0.523$, $p=0.596$).

Figure 4 illustrates in detail the relationship between nectar secretions and TO activity for the lycaenid species with the by far largest amount of data available, viz. *P. icarus*. In experiments with the ant species *Lasius flavus* (Fig. 4a) as well as with *L. niger* (Fig. 4b), prepupal larvae consistently delivered more nectar droplets to attendant ants than feeding caterpillars (Mann–Whitney test; *L. flavus*: $z=14.484$; *L. niger*: $z=7.631$; $p<0.001$). However, while prepupal caterpillars also everted their TO more frequently than feeding larvae during interactions with *L. flavus* ants ($z=4.139$, $p<0.001$), no such increase in TO activity was observed with prepupal caterpillars

Table 2 Main data from the 50 experimental series exploring correlations between tentacle eversion and nectar secretion rate of larvae of nine myrmecophilous *Polyommatinae* species during staged encounters with ants

Series_ID	Lycaenid species	Stage	Nectar delivery (log)	TO eversions (log)	Ant number	Ant species	Interaction intensity	Duration	Pearson's *r*	Sample size	Fisher's *z*	lower CL	upper CL
Exp_1	*Polyommatus icarus*	L4	1.48	1.27	15	*Lasius flavus*	Moderate	Temporary	0.320	69	0.331	0.090	0.572
Exp_2	*Polyommatus icarus*	L4	1.47	1.46	15	*Lasius flavus*	Moderate	Temporary	0.404	103	0.429	0.233	0.625
Exp_3	*Polyommatus icarus*	L4	1.26	1.26	15	*Lasius flavus*	Moderate	Temporary	0.473	132	0.514	0.342	0.687
Exp_4	*Polyommatus icarus*	PP	2.74	1.82	15	*Lasius flavus*	Moderate	Temporary	0.218	124	0.221	0.043	0.399
Exp_5	*Polyommatus icarus*	L3	0.78	1.18	1	*Lasius niger*	Moderate	Temporary	0.767	30	1.012	0.635	1.389
Exp_6	*Polyommatus icarus*	L4	1.09	2.36	1	*Lasius niger*	Moderate	Temporary	0.230	29	0.234	– 0.150	0.619
Exp_7	*Polyommatus icarus*	PP	1.02	1.78	1	*Lasius niger*	Moderate	Temporary	0.652	28	0.778	0.386	1.170
Exp_8	*Polyommatus icarus*	L3	1.19	1.57	5	*Lasius niger*	Moderate	Temporary	0.126	30	0.127	– 0.250	0.504
Exp_9	*Polyommatus icarus*	L4	1.21	2.33	5	*Lasius niger*	Moderate	Temporary	– 0.100	29	– 0.101	– 0.485	0.284
Exp_10	*Polyommatus icarus*	PP	1.78	2.27	5	*Lasius niger*	Moderate	Temporary	0.275	29	0.283	– 0.102	0.667
Exp_11	*Polyommatus icarus*	L3	1.36	1.27	15	*Lasius niger*	Moderate	Temporary	0.201	30	0.204	– 0.174	0.581
Exp_12	*Polyommatus icarus*	L4	1.43	1.62	15	*Lasius niger*	Moderate	Temporary	0.380	125	0.400	0.223	0.578
Exp_13	*Polyommatus icarus*	PP	1.92	1.34	15	*Lasius niger*	Moderate	Temporary	0.286	110	0.295	0.105	0.484
Exp_14	*Polyommatus cornelia*	L4	0.68	1.34	15	*Lasius flavus*	Moderate	Temporary	0.652	12	0.779	0.125	1.432
Exp_15	*Polyommatus cornelia*	L5	1.37	0.85	15	*Lasius flavus*	Moderate	Temporary	0.325	10	0.337	– 0.403	1.078
Exp_16	*Polyommatus cornelia*	PP	2.35	0.00	15	*Lasius flavus*	Moderate	Temporary	0.000	6	0.000	– 1.132	1.132
Exp_17	*Polyommatus amandus*	L4	1.72	0.21	15	*Lasius flavus*	Moderate	Temporary	0.315	10	0.326	– 0.415	1.066
Exp_18	*Polyommatus amandus*	PP	2.20	0.64	15	*Lasius flavus*	Moderate	Temporary	0.576	10	0.657	– 0.084	1.398
Exp_19	*Polyommatus amandus*	L3	1.50	0.00	15	*Lasius niger*	Moderate	Temporary	0.125	19	0.126	– 0.364	0.616
Exp_20	*Polyommatus amandus*	L4	0.35	0.48	15	*Lasius niger*	Moderate	Temporary	– 0.145	48	– 0.146	– 0.439	0.146
Exp_21	*Polyommatus amandus*	PP	0.90	1.18	15	*Lasius niger*	Moderate	Temporary	– 0.324	44	– 0.336	– 0.642	– 0.030
Exp_22	*Lysandra coridon*	L3	1.63	2.97	15	*Lasius niger*	Intimate	Temporary	– 0.307	11	– 0.317	– 1.010	0.376
Exp_23	*Lysandra coridon*	L4	1.42	3.09	15	*Lasius flavus*	Intimate	Temporary	0.153	32	0.154	– 0.210	0.518
Exp_24	*Lysandra coridon*	PP	2.31	3.61	15	*Lasius flavus*	Intimate	Temporary	0.178	12	0.180	– 0.473	0.833
Exp_25	*Lysandra bellargus*	L4	1.18	3.41	10	*Lasius flavus*	Intimate	Temporary	– 0.055	11	– 0.055	– 0.748	0.638
Exp_26	*Aricia agestis*	L4	0.10	0.89	1	*Lasius flavus*	Intimate	Permanent	0.378	16	0.398	– 0.146	0.942
Exp_27	*Aricia agestis*	PP	0.32	1.21	1	*Lasius flavus*	Intimate	Permanent	0.409	20	0.435	– 0.041	0.910
Exp_28	*Aricia agestis*	L4	0.26	1.14	2	*Lasius flavus*	Intimate	Permanent	0.679	14	0.828	0.237	1.419
Exp_29	*Aricia agestis*	PP	0.69	1.69	2	*Lasius flavus*	Intimate	Permanent	0.116	19	0.117	– 0.373	0.607
Exp_30	*Aricia agestis*	L4	0.30	1.41	5	*Lasius flavus*	Intimate	Permanent	0.592	10	0.681	– 0.059	1.422
Exp_31	*Aricia agestis*	PP	0.58	1.56	5	*Lasius flavus*	Intimate	Permanent	– 0.262	15	– 0.268	– 0.834	0.298
Exp_32	*Aricia agestis*	L4	0.23	1.28	10	*Lasius flavus*	Intimate	Permanent	0.588	13	0.674	0.055	1.294
Exp_33	*Aricia agestis*	PP	0.57	1.43	10	*Lasius flavus*	Intimate	Permanent	0.119	20	0.119	– 0.356	0.595

Table 2 (continued)

Series_ID	Lycaenid species	Stage	Nectar delivery (log)	TO eversions (log)	Ant number	Ant species	Interaction intensity	Duration	Pearson's r	Sample size	Fisher's z	lower CL	upper CL
Exp_34	*Aricia agestis*	L4	0.93	1.47	5	*Lasius flavus*	Intimate	Temporary	0.266	14	0.272	– 0.319	0.863
Exp_35	*Aricia agestis*	PP	1.21	1.79	5	*Lasius flavus*	Intimate	Temporary	0.375	16	0.394	– 0.150	0.938
Exp_36	*Aricia agestis*	L4	0.82	1.36	10	*Lasius flavus*	Intimate	Temporary	0.711	14	0.888	0.297	1.479
Exp_37	*Aricia agestis*	PP	0.97	1.69	10	*Lasius flavus*	Intimate	Temporary	– 0.033	19	– 0.033	– 0.523	0.457
Exp_38	*Aricia agestis*	L4	1.36	3.48	10	*Lasius flavus*	Intimate	Temporary	– 0.014	13	– 0.014	– 0.634	0.605
Exp_39	*Glaucopsyche alexis*	L4	0.59	2.77	10	*Lasius flavus*	Intimate	Temporary	– 0.472	15	– 0.512	– 1.078	0.054
Exp_40	*Zizeeria knysna*	L4	1.02	2.03	5	*Lasius flavus*	Moderate	Temporary	– 0.135	19	– 0.136	– 0.626	0.354
Exp_41	*Zizeeria knysna*	PP	1.79	2.08	5	*Lasius flavus*	Moderate	Temporary	– 0.265	18	– 0.271	– 0.777	0.235
Exp_42	*Zizeeria knysna*	L4	1.36	1.15	15	*Lasius flavus*	Moderate	Temporary	– 0.055	19	– 0.055	– 0.545	0.435
Exp_43	*Zizeeria knysna*	PP	2.06	1.65	15	*Lasius flavus*	Moderate	Temporary	– 0.420	19	– 0.448	– 0.938	0.042
Exp_44	*Zizeeria knysna*	L3	0.63	0.66	15	*Lasius flavus*	Moderate	Temporary	0.047	30	0.047	– 0.330	0.424
Exp_45	*Zizeeria knysna*	L4	0.80	1.21	15	*Lasius flavus*	Moderate	Temporary	0.537	39	0.600	0.274	0.927
Exp_46	*Zizeeria knysna*	PP	1.05	1.33	15	*Lasius flavus*	Moderate	Temporary	0.225	36	0.229	– 0.113	0.570
Exp_47	*Zizeeria knysna*	L4	0.63	0.75	5	*Myrmica rubra*	Moderate	Temporary	0.583	15	0.667	0.101	1.233
Exp_48	*Zizeeria knysna*	PP	0.89	0.99	5	*Myrmica rubra*	Moderate	Temporary	0.282	15	0.289	– 0.276	0.855
Exp_49	*Celastrina argiolus*	L4	0.60	0.76	15	*Lasius flavus*	Moderate	Temporary	0.302	27	0.311	– 0.089	0.711
Exp_50	*Celastrina argiolus*	PP	1.10	0.46	15	*Lasius flavus*	Moderate	Temporary	– 0.177	13	– 0.179	– 0.799	0.441

Given are the mean frequencies of nectar secretions and TO eversions (both log-transformed), Pearson's correlation r between these two frequencies within each series, the number of replicates in each series, and Fisher's z (and its lower and upper 95% confidence limits CL) as effect size measure. Stage denotes larval instar (L3, L4, L5) or if final instar caterpillars were already in the short non-feeding prepupal stage (PP)

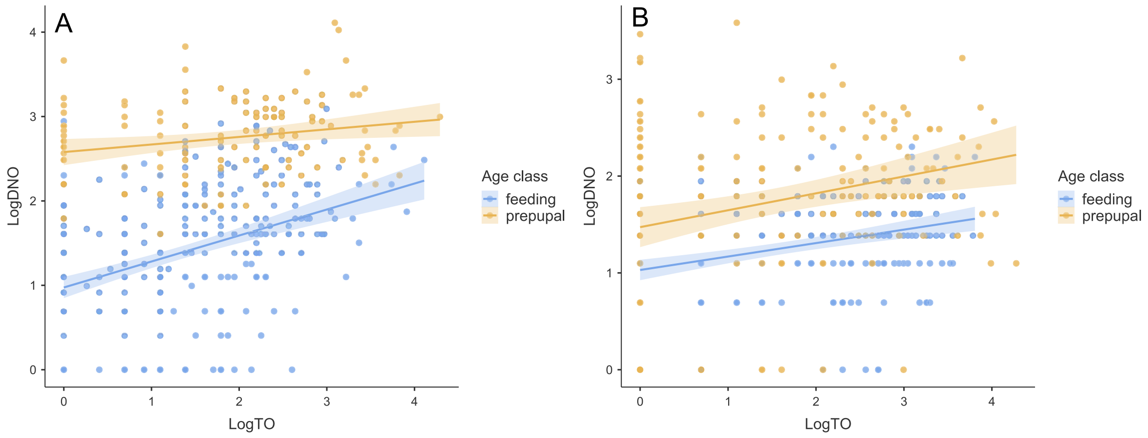

Fig. 4 Scatterplots illustrating the relationship between nectar secretion (DNO) and tentacle (TO) eversion frequency (both on a log-scale) in experiments with caterpillars of *Polyommatus icarus*. Each dot represents the outcome of one experimentally staged encounter (15 min). A: with *Lasius flavus* ants; B: with *L. niger* ants. Non-feeding prepupal caterpillars (brown; n = 124 with *L. flavus* and 167 with *L. niger*) usually delivered more nectar droplets than caterpillars at younger developmental ages (blue; n = 304 with *L. flavus* and 273 with *L. niger*). The relationship between the activity of both myrmecophilous organs was positive, but weak in all cases. For visual clarity, OLS regression lines and their 95% confidence limits are included

in association with *L. niger* ants ($z = 1.008$, $p = 0.843$). Rather, mean as well as median TO eversion frequencies even slightly (but not significantly) decreased in the prepupal phase. The strength of the relationship between nectar delivery and TO eversion rates across caterpillar individuals did not systematically vary according to developmental age of the caterpillars (Mann–Whitney test on effect sizes: $z = 0.077$, $p = 0.939$), nor between both attendant ant species ($z = 0.694$, $p = 0.487$). 208 individuals (24.0%) of *P. icarus* never everted their TO during assays, but only 46 of these 'TO-mute' individuals did also not deliver any single nectar droplet during the 15 min periods of observation. The same caterpillars also everted their TO on other occasions (e.g., during handling) which indicates that their organs were indeed functional.

Results with the other Polyommatinae species were qualitatively similar. Larvae of *Aricia agestis* showed particularly high TO activity (on average 30–60 eversions per caterpillar and trial), but still nectar secretion rates varied widely between tested individuals. Only 18 individuals (9.5%) were 'TO-mute' during experiments. Seventeen *A. agestis* larvae everted their TO even more than 100 times in 30 min (maximum: 276 eversions). Yet, when *A. agestis* caterpillars were grouped into quartiles following their TO eversions, individuals in the uppermost quartile (mean ± 1 SE; 8.48 ± 1.34 droplets/30 min at 70−276 TO eversions) were not significantly more prolific in delivering nectar than those in the second quartile (5.59 ± 0.97 droplets/30 min at 35−67 TO eversions; Dunn's post hoc test: $z = 1.196$, $p = 0.232$, following a Kruskal–Wallis ANOVA across all four quartiles with $H_3 = 31.06$, $p < 0.0001$).

Comparisons across species and experimental series

Across the 50 experimental series, mean DNO secretion rate correlated strongly with mean TO eversion rate in staged encounters using caterpillars of strongly ant-attended species (Fig. 5; $r^2 = 0.6784$, $p < 0.0001$). In contrast, such a relationship was totally absent among experimental series involving caterpillars of moderately myrmecophilous species

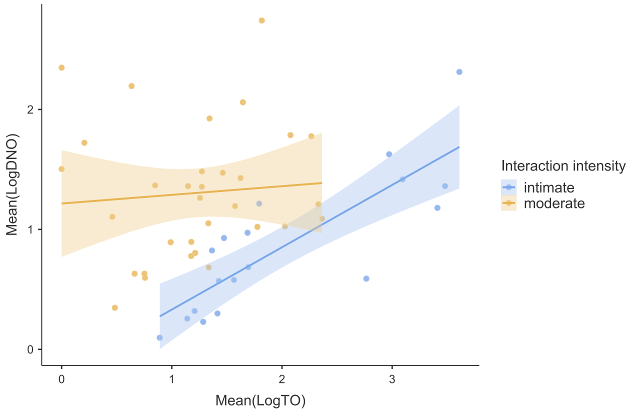

Fig. 5 Scatterplot of average nectar secretion versus mean TO eversion rate (both log-transformed), segregated between Polyommatinae species with intimate (blue) or moderate (brown) ant associations. Each dot represents the mean values observed in one of the 50 experimental series. In intimately ant-attended species (i.e., *Lysandra bellargus, L. coridon, Aricia agestis* and *Glaucopsyche alexis*) activity of both myrmecophilous organs co-varied significantly; this was not observed in experiments using caterpillars of species with lower levels of ant-attendance (*Polyommatus icarus, P. cornelia, P. amandus, Celastrina argiolus*, and *Zizeeria knysna*). For visual clarity, OLS regression lines and their 95% confidence limits are included

($r^2 = 0.0069$, $p > 0.6$). Rather, among these latter experimental series a very large scatter was observed.

Discussion

Covariance between tentacle organ eversions and nectar secretions within and across species

If TO eversions reliably signal the profitability of a lycaenid caterpillar as nectar source to attendant ants, one should expect within as well as across species activity of both myrmecophilous organs to be positively related to each other. So far, evidence in support of that idea mainly came from experiments conducted with one single species, the Palaearctic common blue butterfly *P. icarus* (Leimar and Axén 1993; Axén et al. 1996; Burghardt and Fiedler 1996b).

The data summarized in the present study confirm that within and across species and social conditions such a positive relationship between TO eversions and nectar secretions indeed holds true. Yet, the overall effect size was very low. Across species and conditions, variation in TO activity explained on average less than 7% of variation in nectar delivery rate. Hence, it seems doubtful to what extent TO eversions could indicate the profitability of a caterpillar as a nectar source to attendant ants.

Larvae of the intimately ant-associated *L. coridon* and *L. bellargus* also everted their TO frequently (on average about 30 times per 15 min as mature larvae and 50 times as prepupa). Yet, DNO secretion rates in *L. coridon* and *L. bellargus* did not exceed the range observed in *P. icarus*. Correlations between TO and DNO activity were always low (Fisher's $z < 0.20$) and partly even negative in sign. Hence, also in these closely ant-attended caterpillars the frequency of TO eversions hardly conferred any reliable information to attendant ants about their nectar secretion capacity.

Across species and experimental series, a clear positive relationship between average DNO secretion frequencies and mean TO eversion rates emerged only in experiments with intimately ant-attended caterpillars. Here, a high average activity of the TOs might indicate a higher likelihood of obtaining nectar rewards to visiting ants. In contrast, among moderately to loosely ant-attended cases no relationship between average values per trial series was observed at all, even though especially during the short prepupal phase mean nectar secretion rates were as high or even higher than in intimate myrmecophiles.

Altogether, these experimental data suggest that within as well as across the tested Polyommatinae species nectar secretions and tentacle eversions represent two largely independent behaviours, which may weakly covary in some cases, but not universally so.

The possible role of TO in butterfly–ant communication

Communication between organisms frequently involves multiple signal types (Bro-Jørgensen 2010), usually including receiver manipulation to some extent (Stegmann 2013). Manipulative communication is particularly prevalent among myrmecophiles that mimic signals of their host ants (Hölldobler and Kwapich 2022). Malicky's (1969) early conclusion that communication between lycaenid larvae and ants is largely based on signal copies the caterpillars produce in order to 'deceive' ants has also received multiple support in more recent studies (e.g., Hojo et al. 2009; Thomas et al. 2013; Pierce and Dankowicz 2022a). Moreover, lycaenid larvae employ a range of behavioural strategies to minimize costs and at the same time increase potential benefits accruing from ant attendance, which emphasizes the inherent conflict of interest between the partners.

An alternative, more inclusive interpretation of the function of TO in lycaenid–ant communication seems therefore desirable. I suggest that, by everting their TO, Polyommatinae larvae manipulate the behaviour of attendant ants independently of their nectar secretion propensity. This contributes to their multimodal communication with ants which is mostly shaped by selection to benefit the butterfly side (Fiedler et al. 1996; Pierce and Dankowicz 2022a). Indeed, in all interacting pairs of species tested during this present study, attendant ants frequently responded to TO eversions of the caterpillars by becoming alerted and vigilant (see Fiedler and Maschwitz 1988; Ballmer and Pratt 1991; Ballmer 2022).

If TO eversions and nectar secretions can be varied independently by the caterpillars, then two particular sets of strategies might be selected for. Either caterpillars engage more strongly in manipulative communication, e.g., using TO signals and cuticular odours, instead of offering more food rewards to their visitors. This would be expected in lycaenids with intimate ant associations mediated by high nectar quality (e.g., Daniels et al. 2005) and whose cuticular odours well match the ants' templates. Under this strategy nectar secretions should only be delivered when needed, but largely independent of TO activity. Concomitantly, TO activity should generally be high in these species to keep ants alert and in attendance. This is what the more strongly myrmecophilous test species (*L. coridon, L. bellargus, A. agestis, G. alexis*) did in my experiments. This also implies that TO activity is not very costly to the caterpillars—whatever the chemical or physical nature of their 'signals' might be.

Alternatively, in species with loose ant associations (such as *P. icarus, P. amandus, P. cornelia, Z. knysna, C. argiolus*) caterpillars should keep both their efforts in regard to nectar secretions and TO eversions low to minimize costs.

Investment in myrmecophily should just suffice to retain the ants' attitude as non-hostile. Nectar quality, and accordingly cost, is also lower in these cases (Daniels et al. 2005). However, situations may emerge when it is temporarily advantageous for such a caterpillar to simultaneously maximize investment into nectar delivery and TO activity to achieve optimal ant tending levels. This could result in momentary cases of 'apparent honesty' of signals, emerging only under specific conditions.

For example, if a caterpillar is attacked by an enemy and has the potential to temporarily further increase its level of actual ant attendance, it should activate all possible communication channels in concert to achieve this goal. The concomitant increase of DNO secretions and TO eversions under simulated attacks (Leimar and Axén 1993) fits to this perception, as do the higher activities of both types of myrmecophilous organs at the onset of newly established ant associations (Fiedler and Hagemann 1995; Burghardt and Fiedler 1996b). Following simulated attacks, myrmecophilous butterfly caterpillars also increase their vibratory activities (DeVries 1990; K. Fiedler, unpublished observations), lending further support to the idea that under immediate threat all available communication channels are employed in concert.

Likely, the 'meaning' of TO eversions as part of the multimodal communication system between myrmecophilous lycaenid larvae and ants varies across the clades of the Lycaenidae, since also morphologically these organs are by no means homogeneous (Fig. 1). Interspecific differences as reported here thus might reflect underlying differences in the physical or chemical nature of the still unknown stimuli dissipated by TO eversions. However, it seems unlikely that variation among species as closely related as the six representatives of the subtribe Polyommatina, whose TO are almost indistinguishable morphologically, were caused by radically different stimuli provided by these organs.

If TO manipulate the ants' behaviour, can this be an evolutionarily stable strategy? Two lines of evidence suggest there is little leverage for counter-selection. First, as long as caterpillars continue to deliver at least some nectar, even high TO eversion rates would only marginally increase nectar sampling costs for attendant ants (through increased locomotion activity when alerted), in the sense of biological markets between the two trader classes (Noë and Hammerstein 1994, 1995). It seems unlikely that these low extra costs for a few ant foragers visiting myrmecophilous caterpillars would translate into a substantial selective disadvantage on the ant colony level as selective unit (Nowak et al. 2010). Sizeable sampling costs would only emerge if caterpillars were able to attract ants for extended periods of time using their TO but without rewarding them with any nectar.

Second, many myrmecophiles exploit signals that are essential for the social cohesion of ant societies (Hölldobler and Kwapich 2022). Fitness of sterile worker ants totally depends on their efficiency to contribute to these social tasks. Therefore, myrmecophiles producing signal copies that are sufficiently close to the ants' template escape counter-selection by their visitors, as long as the reproductive success of the ant colony as a whole remains unaffected.

Conclusions

Evidence from over 1500 staged encounters between larvae of nine Palaearctic lycaenid butterfly species and ants questions the view that TO provide reliable signals in mutualistic butterfly–ant associations. TO eversions and nectar secretions often vary independently from another. Within species, there is tremendous variation with regard to nectar secretion rates, but even more so concerning TO eversion frequencies. This renders it almost impossible for foraging ants to assess the profitability of a caterpillar as nectar resource if relying on its TO display. Across species, variation becomes even larger. Therefore, TO do not tell much about an individual caterpillar's capacity to deliver nectar secretion, but rather provide another complementary dimension of multimodal communication between lycaenid larvae and ants. This multi-channel communication ensures that, under a wide range of conditions, lycaenid larvae are able to appease ants and benefit from associations whenever achievable. In more intimate butterfly–ant associations, manipulation of ants is more pronounced and TO eversions occur more frequently. In loosely ant-attended species, in contrast, TO display is overall less frequent. Between these two extremes, species with moderate degrees of ant association can be found where, under certain conditions, DNO and TO activities may co-vary. Yet, even in these apparent cases of reliable signalling caterpillars just use the best selfish strategy to optimize their ant association by manipulating the ants' behaviour.

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Data availability All data needed to re-compute the analyses shown in this paper are contained in Table 2. Raw data from the 1500+ staged encounters can be obtained upon request from the author.

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