Evol Ecol (2011) 25:1205–1216
DOI 10.1007/s10682-011-9478-6

ORIGINAL PAPER

Evolution of influence: signaling in a lycaenid-ant interaction

Jeffrey C. Oliver · Laura R. Stein

Received: 3 September 2010 / Accepted: 22 March 2011 / Published online: 3 April 2011

Abstract Some phytophagous insects gain defense from natural enemies by associating with otherwise potentially harmful top predators. Many lycaenid butterfly caterpillars are involved in such interactions with ants: larvae provide carbohydrate rewards from the dorsal nectary organ (DNO) to associated ants in return for protection from natural enemies. The stability of these interactions involves signals that identify the lycaenid caterpillar as a mutualist. However, larvae of some lycaenid species, such as *Lycaena xanthoides*, are found in close association with ants but do not possess the reward producing DNO. Evaluating the relationship in a phylogenetic framework, we show that the association between *L. xanthoides* and ants likely evolved from a non-ant-associated ancestor. Behavioral trials also show that *L. xanthoides* larvae are capable of influencing ant behavior to increase ant tending when faced with a simulated predator attack, without providing DNO-derived rewards to ant associates. These results demonstrate that the DNO is not necessary to maintain associations between lycaenid larvae and ants. Third-party

---

Electronic supplementary material The online version of this article (doi:10.1007/s10682-011-9478-6) contains supplementary material, which is available to authorized users.

J. C. Oliver (✉)
Department of Ecology and Evolutionary Biology, Yale University,
358 ESC, 21 Sachem Street, New Haven, CT 06520, USA
e-mail: jeffrey.oliver@yale.edu

J. C. Oliver
Interdisciplinary Program in Insect Science, University of Arizona,
1007 E. Lowell Street, Tucson,
AZ 85721, USA

L. R. Stein
Department of Ecology and Evolutionary Biology, University of Arizona,
1041 E. Lowell Street, Tucson, AZ 85721, USA

Present Address:
L. R. Stein
Department of Animal Biology, University of Illinois, 505 S. Goodwin Avenue,
Urbana, IL 61801, USA

interactions may affect the evolution of mutualisms and consideration of underlying evolutionary history is necessary to understand contemporary species associations.

Keywords Mutualism · Myrmecophily · Myrmecoxeny · Character evolution · *Lycaena*

Introduction

Insects have evolved an array of intrinsic physical, behavioral, and chemical defenses to reduce mortality from predators and parasitoids (Pasteels et al. 1983; Gross 1993). Other insect species have adopted an intuitively risky strategy of living in close contact with a top predator, shifting the burden of defense to their partner species (Nault et al. 1976; Pierce and Mead 1981; Weeks 2003). To maintain this indirect defense mutualism, vulnerable partners are equipped with a diverse set of adaptations to keep from becoming prey to their defenders (Kitching and Luke 1985; Stadler and Dixon 2008). As incentive to their protective partners, phytophagous insects often provide nutritional rewards in return for defense from natural enemies (Pierce and Mead 1981; Stadler and Dixon 2005).

Ants are commonly recruited defenders for phytophagous insects, including aphids, cynipid wasps, and lycaenid butterfly caterpillars (Gross 1993). A majority of lycaenid species are involved in mutualistic interactions with ants (Osborn and Jaffé 1997; Pierce et al. 2002): ants respond to signals emitted by larvae, and are provided with a nutritional reward (Pierce and Mead 1981; Fiedler and Maschwitz 1989; Agarwal and Fordyce 2000; Saarinen 2006). These rewards are hypothesized to maintain a "standing guard" of ants, which actively protect larvae from natural enemies (Pierce and Mead 1981). Nutritional rewards can mediate the amount of protection larvae receive from ants (Leimar and Axén 1993; Agarwal and Fordyce 2000), and larvae lacking such rewarding systems should not have the means to recruit protectors (Atsatt 1981). In some cases, commensal lycaenid larvae may gain access to enemy free (ant-patrolled) space without providing nutritional rewards (Osborn and Jaffé 1997); however, in these systems, the larvae do not actively recruit ants, but simply reduce ant aggression. Nutritional rewards thus appear necessary in order to maintain the mutualism between larvae and ants.

Primary in lycaenid-ant mutualisms are the rewards provided by the dorsal nectary organ (DNO; Newcomer 1912; Fiedler and Maschwitz 1989; Leimar and Axén 1993; Pierce et al. 2002). This exocrine gland is used by lycaenid larvae to provide ants with nutritive rewards, including carbohydrates and/or amino acids (Maschwitz et al. 1975; Daniels 2004). Ant attendance is directly related to amount of DNO secretions provided by larvae (Fiedler and Maschwitz 1989; Leimar and Axén 1993; Agarwal and Fordyce 2000) and these rewards have quantitative benefits to ants (Cushman et al. 1994; Fiedler and Saam 1995). DNO-possessing larvae under simulated attacks produce signals which advertise their profitability to ants; these signals alter ant behavior, and ants gain DNO-derived nutritive rewards in return for protection from natural enemies (Leimar and Axén 1993; Agarwal and Fordyce 2000). Although non-rewarding parasitic entomophagic lycaenid larvae are known to manipulate the behavior of their ant hosts (Akino et al. 1999) and *Jalmenus evagoras* Donovan pupae (which lack the DNO) are attractive to ants (Pierce 1983), there is no quantitative evidence of phytophagous DNO-lacking lycaenid larvae influencing ant behavior to gain active ant protection from natural enemies.

In this study, we investigate a system in which larvae lack the reward-producing dorsal nectary organ but are associated with ants. Four species of *Lycaena* (Lepidoptera: Lycaenidae) in the subgenus *Gaeides* (*L. xanthoides* Boisduval, *L. editha* Mead, *L. dione*

Scudder, *L. rubidus* Behr) are all known to associate with ants in the field (Ballmer and Pratt 1991; Allen et al. 2005), but lack the rewarding DNO (Ballmer and Pratt 1988). *Lycaena xanthoides* larvae are known to associate with two ant species, *Formica francoeuri* Bolton and *Liometopum occidentale* Emery and face strong pressure from natural enemies (Ballmer and Pratt 1991; Oliver et al. 2007). Our first goal was to investigate the evolution of the association between DNO-lacking larvae and ants to determine how this association evolved. By inferring the phylogeny of the genus *Lycaena*, and reconstructing ancestral states of ant-association, we can distinguish between two hypotheses: (1) *L. xanthoides* and related *Gaeides* species evolved from a rewarding ant-associated ancestor and subsequently lost the ability to reward tending ants, or (2) the ancestor was not associated with ants, but evolved a mechanism to alter ant behavior without the DNO. The former case would reflect evolution of a "cheating" strategy, where one member of the mutualism ceases to reciprocate while still reaping benefits from its one-time partner. The latter case represents evolution of an association which may or may not rely on stable interactions between the protector species and other rewarding prey species.

Our second goal was to determine if *L. xanthoides* larvae could influence ant behavior to gain protection from natural enemies. Increased ant attendance and/or ant activity would provide more protection from enemies than a commensal interaction, in which ants do not actively respond to signals emitted by the larvae. It is necessary to understand the type of relationship in order to assess the importance of the DNO in lycaenid-ant interactions.

Materials and methods

Natural history

Lycaena xanthoides occupies mesic habitats in low to middle elevations of California, northern Baja, Mexico, and southern Oregon. Larvae feed on 4–5 species of *Rumex* (Polygonaceae) (Scott 1986; Ballmer and Pratt 1988) and are usually associated with ants (Ballmer and Pratt 1991; Oliver et al. 2007). *Formica francoeuri* occupies the mountains of northern Baja, Mexico, and the Transverse and southern Coast ranges of California (Francoeur 1973). *Formica francoeuri* is known to tend larvae of at least six species of lycaenids in California: *Lycaena xanthoides*, *L. heteronea* Boisduval, *Plebejus acmon* Westwood & Hewitson, *P. lupini* Boisduval, *Glaucopsyche piasus* Boisduval, and *Plebulina emigdionis* Grinnell (Ballmer and Pratt 1991). Neither *L. xanthoides* nor *L. heteronea* (subfamily Lycaeninae) larvae possess a DNO; the larvae of the latter four species (all in the subfamily Polyommatinae) all possess a DNO (Ballmer and Pratt 1988).

Evolution of association

To investigate the evolutionary history of *L. xanthoides*' association with ants, we reconstructed a molecular phylogeny of the genus *Lycaena*. Our taxon sampling of the genus *Lycaena* included 21 of approximately 53 species (Hodges et al. 1983; Bozano and Weidenhoffer 2001), including representitives of all seven North American subgenera (Hodges et al. 1983) and five of the six Palearctic species groups of Bozano and Weidenhoffer (2001). We included all four species in the subgenus *Gaeides*, to which *L. xanthoides* belongs. We sequenced three genes: 633–2,021 base pairs of the mitochondrial genes cytochrome oxidase subunits I and II (COI and COII, respectively) and 560–1,195 base pairs of the nuclear gene elongation factor-1 alpha (EF1α). Using the primers Ron, Nancy, Tonya, Hobbes (COI), Pierre, Eva (COII), ef44f, and efrcM4r (EF1α) (Caterino and Sperling 1999; Monteiro and Pierce 2001), all genes were sequenced in both directions on an Applied Biosystems 3730XL DNA Analyzer by the Genomic Analysis and Technology Core (University of Arizona, Tucson, AZ). Consensus sequences were generated and aligned with the aid of phred/phrap (Green 1999; Green and Ewing 2002) and the Chromaseq package of Mesquite (Maddison and Maddison 2007a, b). In addition to specimens sequenced for this study, we also included representatives of six other subfamilies of Lycaenidae, as well as three outgroup species (Table S1; Wahlberg et al. 2005). In all subsequent analyses, COI and COII data were analyzed as single locus, because they are tightly linked on the mitochondrial genome.

To analyze the evolution of ant-association, we performed ancestral character state reconstructions based on (1) independent estimates of gene genealogies for the two loci sequenced and (2) a species-level phylogeny based on the gene genealogies. To estimate the gene genealogies, we performed independent MCMC Bayesian analyses in MrBayes (Ronquist and Huelsenbeck 2003) on the COI/COII and EF1α data. Each gene was partitioned by codon position, and a fourth partition in the COI/COII alignment was assigned to the region coding tRNA-Leucine. All partitions were allowed a unique GTR + G model of evolution. For each locus, we sampled trees every 1,000 generations from two independent MCMC runs of four chains each. Convergence was reached when the average standard deviation of the split frequencies was less than 0.02 (Ronquist and Huelsenbeck 2003). The burnin phases lasted 10 and 30 million generations for the COI/COII and EF1α data, respectively; each analysis was run for an additional 10 million generations following the burnin phase, and only trees sampled in the the final 10 million generations were used for the Bayesian consensus phylogeny and species tree inference (see below). In addition to Bayesian posterior probabilities, we also assessed node support using randomized accelerated maximum likelihood (RAxML, Stamatakis 2006). For each locus, we performed 1,000 bootstrap pseudoreplicates, using a GTR + G model of evolution.

To reconstruct the species-level phylogeny, we used the AUGIST method of Oliver (2008), which uses gene genealogies to reconstruct a species phylogeny based on a criterion of deep coalescences (Maddison 1997; Maddison and Knowles 2006). From the gene genealogy distributions created in gene tree inference analyses, we randomly sampled one gene tree for each locus, and used those two genealogies to infer a species tree. Using Mesquite's Tree Search function (Maddison and Maddison 2007b), we searched for the species tree that minimized the number of deep coalescences of the two contained gene trees. For each search, we employed the subtree pruning-regrafting (SPR) branch swapping algorithm, saving a maximum of ten most optimal trees per search and rooting the trees with *Lycorea halia* Hübner. We repeated this search procedure 200 times, sampling two new gene genealogies for each search. We then generated a consensus species tree using Mesquite's majority rule consensus tree function. The frequency at which a clade occurred in the species tree searches is used as a measure of clade support.

To determine whether *L. xanthoides*' association with ants evolved from an ant-associated or non-ant-associated ancestor, we reconstructed the history of ant association on each of the Bayesian posterior distributions of gene trees and on the distribution of species trees. We coded taxa as being myrmecophilous (ant-associated) or myrmecoxenous (non-ant-associated), based on published records (Table S1). Here we use Pierce et al. (2002) broad definition of myrmecoxeny as non-ant-associated, as opposed to the definition originally offered by Kitching and Luke (1985), which defines taxa as myrmecoxenous if they lack the DNO. Those taxa lacking records regarding ant-association were coded as missing data. For ancestral reconstructions of ant-association on the gene trees, we used the "Trace Over

Trees" function in Mesquite, which reconstructs ancestral history on multiple phylogenies, to incorporate phylogenetic uncertainty in ancestral reconstructions of character states (Maddison and Maddison 2007b). We reconstructed ancestral states on 10,000 randomly-sampled Bayesian post-burnin gene trees using an asymmetrical likelihood model of evolution. Similarly, we estimated ancestral states on 2,000 species trees inferred from Bayesian gene trees using maximum parsimony. These reconstructions were then summarized on the respective consensus tree, where the frequency of each state is reported for each ancestral node. The subgenus *Gaeides* was reconstructed as monophyletic with strong support in gene trees and the estimated species tree (see "Results"), corroborating previous work based on mitochondrial data from a smaller set of taxa (Oliver and Shapiro 2007). For the purposes of ancestral reconstruction, we thus consider myrmecophily of *Gaeides* species to be homologous and focused on nodes within the Lycaeninae clade that are ancestral to *Gaeides*.

To explicitly test the hypothesis that ant-association in *Gaeides* species arose from a non-ant-associated ancestor, we compared two models of evolution. The first model corresponded to a loss-only process of ant-association within the Lycaeninae, where the most recent common ancestor of all Lycaeninae was ant-associated, and ant-association could be lost, but not regained. The second model allowed both gains and losses of ant-association to occur within Lycaeninae. We compared the two models on each of the two posterior distributions of gene trees separately using maximum likelihood estimates of character evolution and on each of the bootstrap samples of RAxML gene trees using maximum parsimony. We also compared the two models on the distribution of species trees based on Bayesian gene tree distributions, using maximum parsimony estimates of ancestral character states. In all analyses, the hypothesis that *Gaeides*' ant-association arose from a non-ant-associated ancestor is supported if the gain/loss model provides a better fit to the observed character data and phylogenetic estimates than a loss-only model.

Laboratory test for influence on ant behavior

Fourth (final) instar *L. xanthoides* larvae and *F. francoeuri* ants were collected in the field from two populations and brought into the laboratory. We collected 5 larvae from Pine Creek (San Diego County, California, 32.8548°N, 116.5228°W) and 4 larvae from Lake Hemet (Riverside County, California, 33.6702°N, 116.6993°W). Each larva was housed singly with host plant material (*Rumex salicifolius* Weinm.) from respective collection site until trials. For trials, a single larva was placed in a container with 9 (Pine Creek) or 14 (Lake Hemet) ants from the same location the larva was collected. We were not testing for population-level differences in ant attendance, so the small difference in the number of ants (9 vs. 14), should not significantly affect our analyses. After a two minute acclimation period, the larva was randomly given one of two treatments: a pinch with forceps on the dorsal thorax ('attack') or no pinch ('control') (Leimar and Axén 1993). In the control treatment, forceps were introduced into the container, above the larva, but no pinch was applied. In the attack treatments, the larval cuticle remained intact, thus avoiding the potential of haemolymph exuding from a wound. The interaction between the larva and ants was video recorded for 5 min. After this trial, the larva was removed from ant container and placed in a container with *Rumex salicifolius* for 4 h before receiving the alternate treatment; ants were alone in the container for at least 20 min following the end of the last trial before another trial began.

To determine if ants responded differently to the different treatments, we recorded the total ant-seconds for each trial. Ant-seconds reflect the amount the larva was tended by each ant. For example, if a larva was tended for 10 s by one ant, and 15 s by another ant, the total for that trial would be 25 ant-seconds. Note that if multiple ants tended a larva at a single time, each of those ants' tending times was included. We tested for within-larvae differences between treatments using a paired t test to determine if tending rates were different between control and attack treatments. Additionally, we measured the total time each larva was moving during the trial to control for a potential effect of movement on our measurement of ant attendance. Greater movement by the larva may have afforded greater opportunity to come into contact with ants, producing an artificial positive relationship between a simulated predation event and our measure of ant tending. We performed another repeated-measures linear regression by including the total time each larva was moving as a covariate, to determine if an effect of treatment was significant even when controlling for a potential relationship between larval movement and our measure of ant tending. All analyses were carried out using the R software package (R Development Core Team 2007).

Results

Evolution of association

Phylogenetic reconstructions of gene trees revealed little resolution among the subfamilies of Lycaenidae (Fig. S1), although our results did not conflict with previous estimates of Lycaenid relationships (Eliot 1973; Pierce et al. 2002). The subfamily Lycaeninae was reconstructed as monophyletic with high posterior probability for both loci and relationships among *Lycaena* species were similar to those of prior studies (Pratt and Wright 2002; van Dorp 2004). The consensus inferred species tree, based on 2,000 trees recovered in the tree-searching procedure, similarly reflects the monophyly of subfamily Lycaeninae, as well as uncertainty in early lycaenid divergences (Fig. S2). The subgenus *Gaeides* was reconstructed as monophyletic with strong support in gene trees and the estimated species tree, corroborating previous work based on mitochondrial data from a smaller set of taxa (Oliver and Shapiro 2007).

Ancestral state reconstructions indicate the association observed in *L. xanthoides* and other members of the subgenus *Gaeides* likely evolved from a myrmecoxenous (non-ant-associated) ancestor. Figure 1 shows estimates of evolution of ant-association within the Lycaeninae. For both gene trees, the nodes within Lycaeninae that are ancestral to the subgenus *Gaeides* are reconstructed as myrmecoxenous with high likelihood (Fig. 1a, b). Ancestral state estimates of ant-association on the species tree distributions corroborate the evolution of ant-association in *Gaeides* from a non-ant-associated ancestor (Fig. 1c).

Explicit tests of the evolution of myrmecophily in *Gaeides* also fail to support a model in which *Gaeides*' association with ants did not evolve from a non-ant-associated ancestor. Loss-only models provided a significantly worse fit to the data than models allowing a *Gaeides*-specific gain of ant-association (COI/COII $\Delta \ln L$ mean $= 24.42$, minimum $= 22.71$; EF1α $\Delta \ln L$ mean $= 29.71$, minimum $= 24.70$). There were no trees in either of the posterior distributions of gene trees in which a loss-only model provided a better fit to observed data. In the bootstrap samples from maximum likelihood estimations of the two gene trees, loss-only models always provided a worse fit than gain/loss models (COI/COII mean difference in steps between loss-only and gain/loss models $= 3.48$; EF1α mean difference in steps $= 3.65$). Similarly, in the sample of 2,000 species trees, loss-only models produced a worse fit than gain/loss models; loss-only models required, on average, 3.6 more steps than gain/loss models. In the sample of 2,000 trees, no trees fit a loss-only model as well or better than a gain/loss model. In both gene tree and species tree

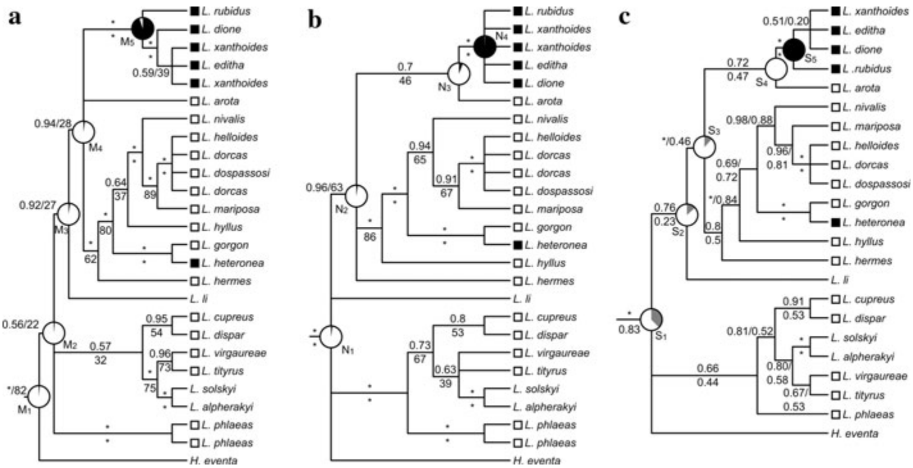


Fig. 1 History of ant association of *L. xanthoides*' ancestors reconstructed on (**a**) COI and COII gene tree, (**b**) EF1α gene tree, and (**c**) species tree. Only Lycaeninae clade of each phylogenetic estimate is shown. In (**a**) and (**b**), *pie-charts on nodes* indicate average relative likelihood of each ancestral state (myrmecoxenous = *white*; myrmecophilous = *black*) reconstructed over 10,000 gene trees; labels above branches represent Bayesian posterior probabilities and labels below branches indicate maximum likelihood bootstrap support. In (**c**), *pie-charts* indicate proportion of 2,000 species trees in which character state was reconstructed for a particular state; branch labels indicate clade frequency in species tree searches based on Bayesian gene trees (*above* branches) or maximum likelihood gene trees (*below* branches). *Grey* indicates proportion of reconstructions in which optimal ancestral state is equivocal between myrmecophily and myrmecoxeny. *Asterisks* (*) indicate branch support ≥0.99 in Bayesian and species tree analyses or branch support ≥90 in maximum likelihood bootstrap analyses

approaches, the analyses incorporated uncertainty in phylogenetic estimates, yet loss-only models were never supported.

Laboratory test for influence on ant behavior

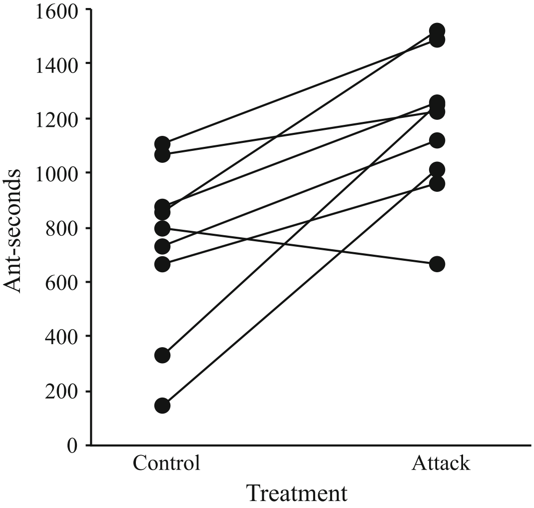
In all trials, ants contacted larvae with forelimbs and antennae, and often antennated the posterior end of the larva. No ant aggression towards larvae was observed in any trial, although *F. francoeuri* workers will attack other non-rewarding lycaenid larvae in a laboratory setting (Ballmer and Pratt 1991). For eight of nine larvae tested, we found higher rates of ant-tending in attack treatments than control treatments (Fig. 2). On average, individual larvae were tended more when subjected to a simulated attack than in control treatments ($t_8 = 3.92$, $P = 0.004$). When controlling for a possible effect of larval movement on ant tending, the ant tending responses remained significantly higher in simulated attack treatments than in control treatments ($F_{1,7} = 7.552$, $P = 0.0286$). These results demonstrate the ability of *L. xanthoides* larvae to influence ant behavior without the DNO-derived rewards.

Discussion

Evolution of association

Both gene tree and species tree reconstructions of the evolution of ant association support the hypothesis that *L. xanthoides* and fellow *Gaeides* species' association with ants arose

Fig. 2 Ant-tending responses to attack and control treatments. *Lines* connect observations in each treatment for individual larvae



from a non-ant-associated ancestor (Fig. 1). In gene tree reconstructions, ancestors of *Gaeides* were reconstructed with relatively high likelihood as being myrmecoxenous. Although some reconstructions of the evolution of ant-association on the estimated species tree were equivocal, potentially due to uncertainty in inferred relationships among members of Lycaeninae (Fig. S2), there is little support for a myrmecophilous ancestor to Lycaeninae species included in this study. Models of loss-only evolution of myrmecophily in Lycaeninae are not supported, further corroborating the hypothesis that the association of *Gaeides*' species with ants has arisen from a myrmecoxenous ancestor. It is important to note that although support for the various ancestral nodes in phylogenetic estimates varied, model evaluation analyses incorporating phylogenetic uncertainty always supported the hypothesis that ant-association was gained in the ancestral lineage giving rise to *Gaeides* species. This origin of ant-association is not surprising given the lability of ant-association in some groups of lycaenids (Megens et al. 2005) and the current understanding of lycaenid phylogenetic relationships, which suggests that ant-association has been gained and lost multiple times within the family (Pierce et al. 2002).

Laboratory test for influence on ant behavior

This interaction between *L. xanthoides* larvae and *F. francoeuri* may represent a case of aggressive chemical mimicry (Wickler 1968; Dettner and Liepert 1994). In aggressive mimicry, a deceiver gains access to resources by mimicking a rewarding model (Wickler 1968; Vane-Wright 1976; Ruxton et al. 2004). In this case, *L. xanthoides* larvae may mimic the signals produced by other, rewarding lycaenid larvae that *F. francoeuri* tends in nature. This interaction may be possible due to *F. francoeuri*'s generalist strategy of tending. In addition to natural associations with at least six species of California lycaenid caterpillars, *F. francoeuri* workers will tend lycaenid species they have never encountered, including at least one species from Asia in laboratory trials (Ballmer and Pratt 1991). The mechanism of signaling employed by *L. xanthoides* also warrants further investigation, especially because *Lycaena* species lack tentacular organs, which may be used by DNO-possessing lycaenid larvae to advertise the DNO-derived nutritive reward (Fiedler et al. 1996).

The phenomenon of chemical mimicry may be more common among DNO-lacking larvae than previously thought, and other potential examples involving lycaenid larvae lacking the rewarding DNO and their ant protectors include *Lycaena dispar* Haworth (Lycaeninae) and *Myrmica laevinodis* Nylander (Myrmicinae) (Hinton 1951), *Curetis regula* Evans (Curetinae) and *Anoplolepis longipes* Jerdon (Formicinae) (DeVries 1984), and *Aloeides dentatis* Swierstra (Theclinae) and *Lepisiota* (*Acantholepis*) *capensis* Mayr (Formicinae) (Henning 1983). This hypothesis of chemical mimicry suggests that the dorsal nectary organ is not proximally required for lycaenid larvae to gain protection by ants, but ultimately necessary (in other co-occurring lycaenid species) for a stable mimetic relationship to persist. In order to confirm this is mimicry of other lycaenid species, it will be necessary to compare the responses of *F. francoeuri* to other, rewarding species of lycaenid larvmae, in experiments analogous to those presented in this study. Furthermore, the signals (chemical, acoustic, or visual) used by *L. xanthoides* and those used by rewarding lycaenid larvae, also require identification to test alternatives to the chemical mimicry hypothesis. Additional explanations for ant-attendance of DNO-lacking species include the possibilities (1) that ants are responding to (presumably injured) larvae as potential prey and (2) that larvae are exploiting a separate pre-existing sensory-bias in ant species, unrelated to signals produced by rewarding lycaenid larvae.

Alternatively, *L. xanthoides* may be providing rewards from other structures, such as the pore cupola organs (PCOs) or dendritic setae (Ballmer and Pratt 1988, 1991; Pierce et al. 2002). Pore cupola organs are epidermal glands present in almost all species of Lycaenidae and are hypothesized to secrete substances to reduce ant aggression (Pierce et al. 2002). Two studies have demonstrated that extracts of the larval dorsal surface epidermis (where PCOs are concentrated) are more attractive to ants than are extracts of the ventral surface in DNO-possessing lycaenid species (*Jalmenus evagoras*: Pierce 1983; *Glaucopsyche lygdamus*: Fiedler et al. 1992). Additionally, extracts of fifth instar *J. evagoras* epidermis contained at least 15 amino acids and in fluorescence stains, o-phthaldehyde (which binds to primary amines, including amino acids) localized to pore cupolas and dorsal setae (Pierce 1983). The functions of dendritic setae are currently unknown, but their presence is correlated with ant associations in lycaenids (Ballmer and Pratt 1988). However, without additional evidence of nutritive rewards from these structures, it is uncertain if they would provide enough incentive to maintain a stable mutualism with attendant ants. In most lycaenid species, the DNO is primarily responsible recruitment and retention of ant attendants (Fiedler and Maschwitz 1989; Leimar and Axén 1993; Agarwal and Fordyce 2000) and the secretions provide a significant nutritive benefit to attendant ants (Cushman et al. 1994; Fiedler and Saam 1995). Therefore, we find it unlikely that *Gaeides* species are emitting honest signals when faced with attack, given the absence of any known rewarding structure.

It is important to note other DNO-lacking lycaenids that influence ant behavior. Parasitic inquilines of ants, such as *Maculinea rebeli* Hirschke, gain access to both food resources and enemy-free space via chemical mimicry of their host ant species (Akino et al. 1999). Although the difference in life history is important (*L. xanthoides* and other *Gaeides* species remain phytophagous throughout their immature development, while late-instar *M. rebeli* are primarily entomophagous), *M. rebeli* use chemical signals to manipulate ant behavior without providing rewards from a dorsal nectary organ. Additionally, the pupae of *J. evagoras* are attractive to ants despite the absence of the DNO in this developmental stage, likely due to amino acids secreted (Pierce 1983) and acoustic signals produced by pupae (Travassos and Pierce 2000). Thus in the pupal stage, *J. evagoras* may still be providing an honest signal to ants in the form of amino acid rewards. These two cases, chemical mimicry by parasitic inquilines and signaling by potentially rewarding pupae, demonstrate the ability of lycaenid species to influence ant behavior even in the absence of the DNO. However, significant differences in life history traits between *M. rebeli* and *L. xanthoides* (parasitic entomophagy versus phytophagy) and *J. evagoras* and *L. xanthoides* (amino acid secreting, once DNO-possessing pupae versus DNO-lacking larvae), indicate qualitatively different interactions between larvae and ants in these cases.

Conclusion

We conclude that *L. xanthoides* larvae influence ant behavior when attacked and this association arose from a non-ant-associated ancestor. This association may represent an example of aggressive mimicry by lycaenid larvae: by emitting signals mimicking those of rewarding, DNO-possessing species of lycaenid larvae, *Gaeides* species may gain indirect defense from attendant ants without providing a nutritive reward. Additional analyses of other morphological structures are necessary to rule out the possibility of alternative reward sources. The mutualism between reward-producing lycaenid larvae and ants may be susceptible to parasitism by other ant species (Fraser et al. 2001), and may also be taken advantage of by non-rewarding lycaenid species.

There may be more than one way to gain indirect defenses from predators and parasites. The well-documented mutualism between reward producing prey and protecting predator provides an opportunity that other prey species may exploit. Such exploitation likely requires specialized adaptations to maintain a stable relationship, and, as evidenced in this study, may arise in relatively unrelated lineages (Bronstein 2001). Although additional morphological and behavioral analyses would improve our understanding of this system, this study highlights the importance of quantitative analyses of species interactions and considerations of other potential players in the evolution of mutualisms (Bronstein 2001; Stanton 2003).

Acknowledgments We thank K.L. Prudic, E.C. Snell-Rood, J.M. Davis, and especially D.R. Papaj for discussions concerning behavioral analyses and species interactions. D.R. Maddison and M.J. Sanderson provided insight on analyses of character evolution. We also thank G. Anweiler, G.C. Bozano, A.M. Shapiro, and E. Weingartner for providing specimens. G.F. Pratt and G.R. Ballmer provided invaluable information regarding field sites and larval rearing conditions. This work was funded by the Center for Insect Science and an NSF DDIG to J.C.O.

References

Agarwal AA, Fordyce JA (2000) Induced indirect defense in a lycaenid-ant association: the regulation of a resource in a mutualism. Proc R Soc Lond B 267:1857–1861

Akino T, Knapp JJ, Thomas JA, Elmes GW (1999) Chemical mimicry and host specificity in the butterfly *Maculinea rebeli*, a social parasite of *Myrmica* ant colonies. Proc R Soc Lond B 266:1419–1426

Allen TJ, Brock JP, Glassberg J (2005) Caterpillars in the field and garden: a field guide to the butterfly caterpillars of North America. Oxford University Press, Oxford

Atsatt PR (1981) Lycaenid butterflies and ants: selection for enemy-free space. Am Nat 118:638–654

Ballmer GR, Pratt GF (1988) A survey of the last instar larvae of the Lycaenidae (Lepidoptera) of California. J Res Lepid 27:1–81

Ballmer GR, Pratt GF (1991) Quantification of ant attendance (myrmecophily) of lycaenid larvae. J Res Lepid 30:95–112

Bozano GC, Weidenhoffer Z (2001) Lycaenidae part I: subfamily Lycaeninae. In: Bozano GC (ed) Guide to the butterflies of the Palearctic region. Omnes Artes, Milan, pp 1–62

Bronstein JL (2001) The exploitation of mutualisms. Ecol Lett 4:277–287

Caterino MS, Sperling FAH (1999) *Papilio* phylogeny based on mitochondrial cytochrome oxidase I and II genes. Mol Phylogenet Evol 11:122–137

Cushman JH, Rashbrook VK, Beattie AJ (1994) Assessing benefits to both participants in a lycaenid-ant association. Ecology 75:1031–1041

Daniels, H. 2004. Facultative butterfly-ant interactions—the role of variation in composition of nectar secretions. Ph.D. dissertation, University of Bayreuth, Bayreuth

Dettner K, Liepert C (1994) Chemical mimicry and camouflage. Annu Rev Entomol 39:129–154

DeVries PJ (1984) Of crazy-ants and Curetinae: are *Curetis* butterflies tended by ants? Zool J Linnean Soc 80:59–66

Eliot JN (1973) The higher classification of the Lycaenidae (Lepidoptera): a tentative arrangement. Bull Brit Mus (Nat Hist), Entomol 28:1–505

Fiedler K, Maschwitz U (1989) Functional analysis of the myrmecophilous relationships between ants (Hymenoptera: Formicidae) and Lycaenids (Lepidoptera: Lycaenidae). Ethology 80:71–80

Fiedler K, Saam C (1995) Ants benefit from attending facultatively myrmecophilous Lycaenidae caterpillars: evidence from a survival study. Oecologia 104:316–322

Fiedler K, Seufert P, Pierce NE, Pearson JG, Baumgarten H-T (1992) Exploitation of lycaenid-ant mutualisms by braconid parasitoids. J Res Lepid 31:153–168

Fiedler K, Hölldobler B, Seufert P (1996) Butterflies and ants: the communicative domain. Experientia 52:14–24

Francoeur A (1973) Revision taxonomique des especes nearctiques du group *fusca*, genre *Formica* (Hymenoptera: Formicidae). Mem Soc Entomol Québec 3:1–316

Fraser AM, Axén AH, Pierce NE (2001) Assessing the quality of different ant species as partners of a myrmecophilous butterfly. Oecologia 129:452–460

Green P (1999) Phrap. http://phrap.org

Green P, Ewing B (2002) Phred. http://phrap.org

Gross P (1993) Insect behavioral and morphological defenses against parasitoids. Ann Rev Entomol 38:251–273

Henning FF (1983) Chemical communication between lycaenid larvae (Lepidoptera: Lycaenidae) and ants (Hymenoptera: Formicidae). J Entomol Soc S Afr 46:341–366

Hinton HE (1951) Myrmecophilous Lycaenidae and other Lepidoptera—a summary. Proc Trans S London Entomol Nat Hist Soc 1949–50:111–175

Hodges RW, Dominick T, Davis DR, Ferguson DC, Franclemont JG, Munroe EG, Powell JA (eds) (1983) Check list of the lepidoptera of America North of Mexico (Including Greenland). E. W. Classey Ltd. and The Wedge Entomological Research Foundation, London

Kitching RL, Luke B (1985) The myrmecophilous organs of the larvae of some British Lycaenidae (Lepidoptera): a comparative study. J Nat Hist 19:259–276

Leimar O, Axén AH (1993) Strategic behaviour in an interspecific mutualism: interactions between lycaenid larvae and ants. Anim Behav 46:1177–1182

Maddison WP (1997) Gene trees in species trees. Syst Biol 46:523–536

Maddison WP, Knowles LL (2006) Inferring phylogeny despite incomplete lineage sorting. Syst Biol 55:21–30

Maddison DR, Maddison WP (2007a) Chromaseq: a Mesquite module for analyzing sequence chromatograms. Version 0.91. http://mesquiteproject.org/packages/chromaseq

Maddison WP, Maddison DR (2007b) Mesquite: a modular system for evolutionary analysis. Version 2.0bi44. http://mesquiteproject.org

Maschwitz U, Wüst M, Schurian K (1975) Bläulingsraupen als Zuckerlieferanten für Ameisen. Oecologia 18:17–21

Megens H-J, de Jong R, Fiedler K (2005) Phylogenetic patterns in larval host plant and ant association of Indo-Australian Arhopalini butterflies (Lycaenidae: Theclinae). Biol J Linn Soc 84:225–241

Monteiro A, Pierce NE (2001) Phylogeny of *Bicyclus* (Lepidoptera: Nymphalidae) inferred from COI, COII and EF-1alpha gene sequences. Mol Phylogenet Evol 18:264–281

Nault LR, Montgomery ME, Bowers WS (1976) Ant-aphid association: role of aphid alarm pheromone. Science 192:1349–1351

Newcomer EJ (1912) Some observations on the relation of ants and lycaenid caterpillars, and a description of the relational organs of the latter. J NY Entomol Soc 20:31–36

Oliver JC (2008) AUGIST: Inferring species trees while accommodating gene tree uncertainty. Bioinformatics 24:2932–2933

Oliver JC, Shapiro AM (2007) Genetic isolation and cryptic variation within the *Lycaena xanthoides* species group (Lepidoptera: Lycaenidae). Mol Ecol 16:4308–4320

Oliver JC, Prudic KL, Pauly GB (2007) Parasitism rates in larval *Lycaena xanthoides* (Godart) (Lepidoptera: Lycaenidae) and a new host record for *Cotesia theclae* (Riley) (Hymenoptera: Braconidae). Pan-Pac Entomol 83:262–264

Osborn F, Jaffé K (1997) Cooperation vs. exploitation: interactions between Lycaenid (Lepidoptera: Lycaenidae) larvae and ants. J Res Lepid 34:69–82

Pasteels JM, Grégoire J-C, Rowell-Rahier M (1983) The chemical ecology of defense in arthropods. Ann Rev Entomol 28:263–289

Pierce NE (1983) Ecology and evolution of symbioses between lycaenid butterflies and ants. Ph.D. thesis, Harvard University, Cambridge, Massachusetts

Pierce NE, Mead PS (1981) Parasitoids as selective agents in the symbiosis between lycaenid butterfly larvae and ants. Science 211:1185–1187

Pierce NE, Braby MF, Heath A, Lohman DJ, Mathew J, Rand DB, Travassos MA (2002) The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). Ann Rev Entomol 47:733–771

Pratt GF, Wright DM (2002) Allozyme phylogeny of North American coppers (Lycaeninae: Lycaenidae). Pan-Pac Entomol 78:219–229

R Development Core Team (2007) R: a language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria) ISBN 3-900051-07-0, URL http://www.R-project.org

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Ruxton GD, Sherratt TN, Speed MP (2004) Avoiding attack: the evolutionary ecology of crypsis, Warning Signals and Mimicry. Oxford University Press, Oxford

Saarinen EV (2006) Differences in worker caste behaviour of *Oecophylla smaragdina* (Hymenoptera: Formicidae) in response to larvae of *Anthene emolus* (Lepidoptera: Lycaenidae). Biol J Linnean Soc 88:391–395

Scott JA (1986) The Butterflies of North America: a natural history and field guide. Stanford University Press, Stanford

Stadler B, Dixon AFG (2005) Ecology and evolution of aphid-ant interactions. Ann Rev Ecol Evol Syst 36:345–372

Stadler B, Dixon AFG (2008) Mutualism: ants and their insect partners. Cambridge University Press, Cambridge

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690

Stanton ML (2003) Interacting guilds: moving beyond the pairwise perspective on mutualisms. Am Nat 162:S10–S23

Travassos MA, Pierce NE (2000) Acoustics, context and function of vibrational signalling in a lycaenid butterfly–ant mutualism. Anim Behav 60:13–26

van Dorp K (2004) Molecular systematics of Lycaena F., 1807 (Lepidoptera: Lycaenidae)—Some preliminary results. Proc Neth Entomol Soc 15:65–70

Vane-Wright RI (1976) A unified classification of mimetic resemblances. Biol J Linnean Soc 8:25–56

Wahlberg N, Braby MF, Brower AVZ, de Jong R, Lee M-M, Nylin S, Pierce NE, Sperling FAH, Vila R, Warren AD, Zakharov E (2005) Synergistic effects of combining morphological and molecular data in resolving the phylogeny of butterflies and skippers. Proc R Soc B 272:1577–1586

Weeks JA (2003) Parasitism and ant protection alter the survival of the lycaenid *Hemiargus isola*. Ecol Entomol 28:228–232

Wickler W (1968) Mimicry in plants and animals. McGraw-Hill Book Company, New York