High host-plant nitrogen content: a prerequisite for the evolution of ant–caterpillar mutualism?

L. PELLISSIER*, S. RASMANN*, G. LITSIOS*†, K. FIEDLER‡, A. DUBUIS*, J. POTTIER* & A. GUISAN*

*Department of Ecology and Evolution, University of Lausanne, Bâtiment Biophore, Lausanne, Switzerland
†Swiss Institute of Bioinformatics, Génopode, Quartier Sorge, Lausanne, Switzerland
‡Department of Animal Biodiversity, University of Vienna, Vienna, Austria

Abstract

The amount of nitrogen required to complete an insect's life cycle may vary greatly among species that have evolved distinct life history traits. Myrmecophilous caterpillars in the Lycaenidae family produce nitrogen-rich exudates from their dorsal glands to attract ants for protection, and this phenomenon has been postulated to shape the caterpillar's host-plant choice. Accordingly, it was postulated that evolution towards myrmecophily in Lycaenidae is correlated with the utilization of nitrogen-rich host plants. Although our results were consistent with the evolutionary shifts towards high-nutrient host plants serving as exaptation for the evolution of myrmecophily in lycaenids, the selection of nitrogen-rich host plants was not confined to lycaenids. Butterfly species in the nonmyrmecophilous family Pieridae also preferred nitrogen-rich host plants. Thus, we conclude that nitrogen is an overall important component in the caterpillar diet, independent of the level of myrmecophily, as nitrogen can enhance the overall insect fitness and survival. However, when nitrogen can be obtained through alternative means, as in socially parasitic lycaenid species feeding on ant brood, the selective pressure for maintaining the use of nutrient-rich host plants is relaxed, enabling the colonization of nitrogen-poor host plants.

Introduction

Caterpillars display highly selective behaviour towards their host plants, as the choice of food sources can affect their fitness, both directly (e.g. growth rate and fecundity) and indirectly (e.g. acquired chemical defence against predators) (Duffey, 1980; Awmack & Leather, 2002). The host-plant choice can be driven by plant traits, such as chemistry, leaf trichomes and nutrient availability (Thompson & Pellmyr, 1991), and from an herbivore's perspective, the suitability of a host plant is influenced by its content of elementary compounds, such as phosphorous, carbon or nitrogen (Huberty & Denno, 2006; Behmer, 2009). For instance, nitrogen has been postulated to

be the major limiting factor for the development of insect herbivores that prefer food plants with a higher nutritive value (Mattson, 1980; Braby, 1994; Awmack & Leather, 2002; Morehouse & Rutowski, 2010). However, the quantity of nitrogen required to complete the life cycle may vary greatly among insect species, particularly in relation to life history traits (Awmack & Leather, 2002). As a consequence, in the absence of other constraints (e.g. plant chemical defences), insects are hypothesized to select food plants that have the highest nitrogen content that supports their life cycle.

Butterfly species display a large variety of life histories, including the unique interaction between the immature stages of many members of the Lycaenidae family (superfamily Papilionoidea) and ants (Hymenoptera: Formicoidea) (Fiedler 1991, Pierce et al., 2002). Species of ant-associated (i.e. myrmecophilous) caterpillars produce nitrogen-rich exudates from their dorsal glands to attract ants (Daniels et al., 2005). In exchange for the nutritious

Correspondence: Loïc Pellissier, Department of Ecology and Evolution, University of Lausanne, Bâtiment Biophore, CH-1015 Lausanne, Switzerland. Tel.: +41 21 692 42 54; fax: +41 21 692 42 65; e-mail: loic.pellissier@unil.ch

secretions they obtain, the ants do not prey on the caterpillars and, additionally, drive away potential predators and parasitoids (Pierce *et al.*, 2002). However, tremendous variation exists in the strength of butterfly–ant associations, including completely obligatory, loosely facultative and parasitic interactions. In obligatory lycaenid–ant interactions, the caterpillars depend on one or a few specific ant species and suffer excessive mortality when deprived of these ant attendants (Fiedler, 2006). In contrast, facultative myrmecophilous lycaenids do not depend on ant attendance, even though the presence of nonspecific ant visitors may substantially reduce the mortality of the caterpillars (Pierce *et al.*, 2002). Lycaenids that are devoid of nectar glands (myrmecoxenous lycaenids) do not enter into trophobiotic associations with ants but may still avoid ant attack through various communication channels, such as pheromone production (Fiedler *et al.*, 1996; Pierce *et al.*, 2002). Lastly, a small set of lycaenid species are obligate, highly host-specific social parasites of ants (Fiedler, 1998): after oviposition and early larval development on a specific host plant, parasitic caterpillars are adopted into an ant colony, where they feed on the brood (Als *et al.*, 2004; Thomas *et al.*, 2009).

The behavioural range of ants towards lycaenid caterpillars – protective, neutral or, more rarely, predacious – is determined in part by the quality and quantity of rewards the ants receive from these herbivores (Pierce, 1985). These rewards, in turn, may be influenced by the caterpillar host-plant choice because sugars and amino acids allocated to the production of nectar vary depending on the larval host-plant species and quality. Thus, the lycaenid host-plant choice can influence the outcome of the interactions between caterpillars and their attending ants (Pierce 1983, Cushman & Whitham, 1991). For instance, the variation in host-plant quality translated into differences in the nectar secretion capacity and ant attendance in the facultative myrmecophile *Polyommatus icarus* (Fiedler, 1990; Burghardt & Fiedler, 1996). Additionally, in the obligate myrmecophile *Jalmenus evagoras*, larvae attended by ants survived better on fertilized than on unfertilized *Acacia decurrens* plants (Baylis & Pierce, 1991). In multispecies comparisons, Pierce (1985) showed that myrmecophilous species were frequently associated with nitrogen-fixing and/or otherwise supposedly protein-rich host plants. In particular, legumes (Fabaceae) are the most important host-plant taxon of lycaenid butterflies (Fiedler, 1995); despite this importance, the precise role of nitrogen in the host-plant choice of myrmecophilous caterpillars remains a subject of controversy. On the basis of a review of host-plant associations of more than 1000 lycaenid species, Fiedler (1995) argued that the available comparative evidence was insufficient to indicate whether the observed association between legume feeding (Fabaceae family) and myrmecophily is the result of the selection of nitrogen-rich plants or whether this pattern represents host-plant trait conservatism within lycaenids. Moreover, the plant

tissues of the Fabaceae family do not systematically imply that a higher level of nitrogen is accessible to herbivores (Kraiser *et al.*, 2011). As a consequence, Fiedler (1996) advocated that 'only a more detailed analysis of nutrient profiles in a broader range of lycaenid host plants, coupled with a cladistic analysis of the butterflies' phylogeny, will help to resolve this topic'.

In this study, we examined the host-plant nutrient levels of ant-associated and non-ant-associated diurnal butterflies across the western region of the Swiss Alps. In parallel, we measured the leaf traits typically associated with palatability (specific leaf area [SLA], leaf dry matter content [LDMC], and nitrogen [N] and carbon [C] content) of the 250 most frequent and abundant plant species in the study area to characterize the potential nutritional niche available to butterflies. We later collected information on the host-plant affiliations of the butterfly species recorded in the study area. Next, we addressed the following three questions that span three phylogenetic levels: (i) Is the overall trophic niche defined by plant leaf traits (SLA, LDMC, N, C) of butterflies in the Lycaenidae family (which has evolved myrmecophily) distinct from that of the other three families, Nymphalidae, Pieridae and Papilionidae (whose larvae are never myrmecophilous)? (ii) Within the Lycaenidae family, do the butterflies' life histories (nonmyrmecophilous, myrmecoxenous, myrmecophilous and parasitic) reflect their host-plant choice in relation to the nitrogen content? (iii) Within a clade in which myrmecophily is well established (the subfamily Polyommatinae: family Lycaenidae), is the myrmecophily level correlated with the host-plant nitrogen content?

Methods

Study area and field sampling

We observed butterfly and host-plant occurrences in an area of approximately 700 km^2 situated in the western region of the Swiss Alps, ranging from 1000 to 3210 m a.s.l. Plots were chosen outside of forested areas following a balanced random-stratified sampling design (Hirzel & Guisan, 2002) based on the elevation, slope and aspect. The butterfly observations were performed over two consecutive years by sampling 192 plots from June 1 to September 15 of each year during the hours when the butterflies are most active (10:00–17:00 h) and only in good weather conditions. Each plot was visited every three weeks to obtain accurate lists of the regional butterfly fauna and flora. For each visit, we walked through a square area of 50 m × 50 m for 45 min and collected butterflies with a net for identification. Within each plot, we monitored the presence/absence of all of the vascular plant species in a smaller (2 m × 2 m) area.

To obtain indices of the host-plant quality and potential niche space accessible to herbivores, the 250 most frequent and abundant vascular plant species in the study area were

sampled. These plants correspond to the species most likely to be colonized by butterflies and spanned all of the angiosperm plant families in the study area. We measured between 4 and 20 individuals per species in areas with contrasting exposures, slopes and elevation to sample the distribution range of each plant species and evaluate the intraspecific trait variation within the region. We tracked the growing season according to the elevation to sample all of the individuals of a plant species at the same phenological stage. For each individual plant, a fully developed leaf was sampled, readily weighed and scanned for the quantification of the leaf area using the software package ImageJ. To obtain the dry mass, we dried the leaves overnight at 40 °C. The specific leaf area (SLA) was calculated as the ratio of the leaf surface to its dry mass and was expressed in $mm^2 mg^{-1}$. The leaf dry matter content (LDMC) was measured as the ratio of the leaf dry mass to its water-saturated fresh mass (in $mg g^{-1}$) using the partial rehydration method; this measurement is related to the average density of the leaf tissue and tends to correspond to 1/SLA. To measure the leaf carbon (C) and nitrogen (N) contents, ground and dried leaves were analysed by combustion using an elemental analyser.

Butterfly phylogeny

The phylogenetic relationships of all of the butterfly species found in the study area were inferred using DNA sequences obtained from GenBank; species of the family Hesperiidae were included as the outgroup. The sequences included two nuclear (EF1-alpha and Wgl) and four mitochondrial (16s, COI, NDH1 and NDH5) markers. We used MAFFT (Katoh & Toh, 2008; Behmer, 2009) with default settings to align the sequences into a final concatenated matrix of 4,310 base pairs. We applied the Bayesian inference method using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) to reconstruct the phylogenetic tree. MrModeltest was used to select between models of sequence evolution, and for all partitions, the generalized time-reversible model accounting for a gamma distribution was chosen. Six Metropolis-coupled Markov chains within six runs were computed in MrBayes for 10 million generations and were sampled at every 1000th generation starting at different random trees. A burn-in of 7000 sampled generations was applied, and an all-compatible tree was reconstructed with the remaining 3001 trees of each run, that is, 18 000 trees.

Host-plant and myrmecophily data collection

A long tradition of feeding experiments and insect rearing coupled with field observation has permitted us to obtain a good picture of host-plant choice of butterflies, particularly for the well-studied fauna of Central Europe. Using published literature and databases and our own observations (Table S1), for each butterfly species observed, we assembled an exhaustive list of the plant species that are known to serve as host plants for the butterfly larvae in Switzerland and neighbouring regions (Bink, 1992; Braby, 1994, Ebert & Rennwald, 1993; Huemer, 2004; Lepidopterologen-Arbeitsgruppe 1994, LSPN, 1987). For the subsequent analyses, only the host-plant species that occur in the study region were considered. Although not all of the host-plant affiliations have been locally confirmed for the Swiss Alps, this recording allows an estimate of the potential host-plant niche space regionally available for each butterfly species. A total of 25 of the 108 butterfly species were omitted from the analyses, due to the rarity of their host plants. We also collected data from the literature on the myrmecophily traits for all of the butterflies in the study area (Fiedler 1991, Fiedler, 2006).

Multivariate trophic niche comparison of butterfly families

We compared the host-plant trait space used by the most represented butterfly families in the study area, that is, Lycaenidae, Papilionidae, Pieridae and Nymphalidae. The Riodinidae, which is the sister family of the Lycaenidae, was not considered because it contains only one species in Europe. We used the plant traits described above (SLA, LDMC, N, C) to construct the multivariate niche space colonized by the butterflies. We used the framework recently developed by Broennimann et al. (2012) to construct and compare the trophic niche at the butterfly family level, and the trophic niches were compared in a gridded space using a principal component analysis (PCA). First, we run a PCA for the total trophic niche represented by the 250 most frequent and abundant plant species in the study area. In this analysis, each pixel in the environmental space corresponds to a unique combination of leaf traits. Next, the host-plant choice for each butterfly family was projected onto the trophic niche space. Next, we applied a kernel density function to determine the 'smoothened' density of the occurrences of each pixel in the environmental space for each group (Broennimann et al., 2012). We divided the density of occurrences by the density of the environment in each focal pixel to obtain a measure of the density of the host-plant species relative to the availability of its potential nutritional space. This approach has been shown to be notably robust in comparing ecological niches using both real and simulated data sets (Broennimann et al., 2012). The similarity between the trophic niches of the different butterfly families integrating all of the host-plant species records was subsequently measured using Schoener's D, as proposed by Warren et al. (2008), which ranges from 0 (no niche overlap) to 1 (complete overlap). Lastly, an equivalency test was used to test for statistical significance (Warren et al., 2008; Broennimann et al., 2012). The niche equivalency test

determines whether the niches of two families in the trophic niche space are equivalent (i.e. whether the niche overlap is constant when randomly reallocating the association with the host plant among the two families, Broennimann *et al.*, 2012).

Phylogenetic analysis of myrmecophily levels and host-plant nitrogen content

We tested for a phylogenetic pattern that was consistent with the butterflies of different life histories evolving to choose plants with specific nitrogen contents. We computed the phylogenetic signal for the nitrogen content in the host plant using Bloomberg's K. When the evolution of a trait is hypothesized to have been driven by constant selection pressures over time, the Ornstein–Uhlenbeck (OU) model of character evolution (Butler & King, 2004) is a useful alternative to test against a Brownian motion (BM) model that mirrors neutral evolution (Salamin *et al.*, 2010). The OU and BM models are similar in that they incorporate random character drift over time, but unlike BM, the OU models allow traits to move directionally towards selective 'optima' over time. The OU models also allow species (and the internal nodes of the phylogeny) to differ in their optimal trait values based on ecological hypotheses (Butler & King, 2004). Therefore, the OU models are particularly useful when species are hypothesized to be under different selective regimes due to different environmental conditions or ecological scenarios. We separated the butterfly species into five categories based on our field surveys and published accounts: the nonmyrmecophilous category, which includes species of the Pieridae, Papilionidae and Riodinidae; the myrmecoxenous category, which includes species in the Lycaenini and Theclini tribes within the Lycaenidae (Fiedler 1991); and the parasitic category, which includes species from the *Maculinea/Phengaris* clade. The remaining lycaenid species (all in the Polyommatinae) were classified as displaying either high or low myrmecophily according to the strength of the interactions, as in the studies of Baylis & Pierce (1991), Fiedler (2006) and Pellissier *et al.* (in press). On the basis of these categories, we investigated whether different interaction regimes are associated with different optima of nitrogen content in the host plants. The regimes were created by reconstructing the most likely ancestral discrete state for myrmecophily at each node of the tree, which was achieved through the 'ace' function in the APE R package (Paradis *et al.*, 2004) using the default settings. To attribute one value of nitrogen preference to each butterfly species, we computed the average nitrogen content found in their known host-plant species. We fitted the Ornstein–Uhlenbeck (OU) model of character evolution and compared the AIC values of this model against a BM model of trait evolution.

Host-plant nitrogen content and the strength of myrmecophily

Within the Polyommatinae, the species display quantitative interspecific variation in the level of myrmecophily, ranging from a total reduction in ant mutualism (secondary myrmecoxeny) to a completely obligate ant mutualism (high myrmecophily). We investigated whether the evolution of the myrmecophily level is related to the evolution of the host-plant choice in terms of the nitrogen content. Generalized estimating equations (GEEs) implemented in the APE package (Paradis *et al.*, 2004) were used to include the phylogenetic dependence among species. GEEs quantify the dependences of the observations using a correlation matrix based on the phylogenetic relationships between the observed taxa. All of the analyses were performed using R (R Development Core Team 2011).

Results

Trophic niche comparison of butterfly families

When comparing trophic niches of the different butterfly families, we found that the Lycaenidae, Pieridae and Papilionidae families occupy a much more restricted trophic niche compared with the Nymphalidae (Fig. 1, Table 1). Whereas the Nymphalidae have colonized almost all of the trophic space available, Lycaenidae and Pieridae are more restricted to plants with higher nitrogen contents and higher SLAs. In particular, the trophic niches occupied by Lycaenidae and Pieridae are highly similar, as indicated by the high Schoener's D value and the nonsignificant P-value of the equivalency test. In contrast, the niche overlap is much lower when comparing all of the other pairs of families, and the P-values are always significant, indicating nonequivalent niches (Table 1). The family Papilionidae had a low sample size ($n = 4$ species); therefore, the results of this family should be considered cautiously.

Host-plant nitrogen content and myrmecophily

Across all of the butterflies sampled in the study area, we found differences in the level of N content in the host plants eaten by caterpillars that have different degrees of myrmecophily (Figs 2 and 3, one-way ANOVA, $F_{4,75} = 5.2$, $P = 0.001$). Moreover, the phylogenetic analyses showed a significant phylogenetic signal of nitrogen content in the host plants along the butterfly phylogeny ($K = 0.28$, $P = 0.001$), and the OU model with different optima was better able to explain the variance in the host-plant nitrogen content (AIC = 166.9) compared with the BM model (AIC = 179.51). In accordance with the predictions, the Lycaenid species, independent of their myrmecophily level, were associated with the

Fig. 1 Cumulated trophic niche space for 108 species of mountain butterflies of the western region of Switzerland, as defined by the leaf dry matter content (LDC), specific leaf area (SLA), and carbon (C) and nitrogen (N) contents of the larval host plants (see f). (a) Lycaenidae, (b) Nymphalidae, (c) Pieridae and (d) Papilionidae. The niches occupied by all butterflies are also displayed (e). The black line represents the total available (the dashed line the 90th percentile) trophic niche space in the study area defined by the 250 most common angiosperm plant species sampled for leaf traits.
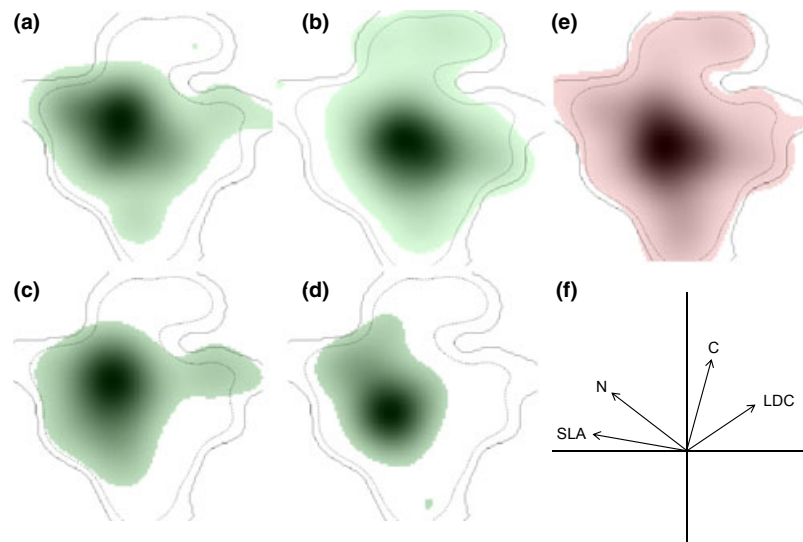
Table 1 Schoener's *D* values and associated *P*-values from a niche equivalency test, comparing the overlap between the larval trophic host-plant niches occupied by the butterfly families. A value of 1 indicates a total niche overlap, whereas a value of 0 indicates no overlap.

	Pieridae	Papilionidae	Nymphalidae
Papilionidae	*D* = 0.52; *P* = 0.01	–	–
Nymphalidae	*D* = 0.35; *P* = 0.01	*D* = 0.26; *P* = 0.01	–
Lycaenidae	*D* = 0.77; *P* = 0.98	*D* = 0.55; *P* = 0.01	*D* = 0.38; *P* = 0.01

plants with the highest nitrogen content ($t = -4.5$, $P < 0.0001$). The myrmecoxenous lycaenids and non-myrmecophilous species from the Pieridae family were also found to be associated with host plants having high nitrogen contents.

Host-plant nitrogen content and the strength of myrmecophily

In contrast to the aforementioned family-level comparison, we found no significant relationship between the strength of myrmecophily and the host-plant nitrogen content within the subfamily Polyommatinae once the phylogenetic dependency was accounted for ($t = 2.25$, $P = 0.109$, phylogenetic df = 5, Fig. 4). The modest fraction of variance explained by this relationship ($R^2 = 0.1$) mostly originated from two species: the secondary myrmecoxene high-elevation alpine *Plebejus glandon* and the weakly ant-attended and highly polyphagous *Celastrina argiolus*.

Discussion

Interactions with ants are associated with the cost of providing nitrogen-rich nourishment to the ants, and this association was hypothesized to influence the choice of host plant in Lycaenidae towards more nitrogen-rich plants (e.g. nitrogen-fixing legumes, Pierce, 1985). In the present study, we found that the butterfly species in the Lycaenidae family – both myrmecophilous and myrmecoxenous – do effectively prefer plants with higher nitrogen contents compared to species in the closely related but nonmyrmecophilous Nymphalidae family. Lycaenidae are not unique among butterflies with regard to host-plant choice: the nonmyrmecophilous Pieridae also display a preference for nitrogen-rich plants. Feeding on nitrogen-rich host plants may also provide significant fitness advantages for nonmyrmecophilous species in addition to mymecophilous ones. Ant-parasitic lycaenids that obtain nitrogen by feeding on ant larvae are relaxed from host-plant specific selection and can feed on plants with reduced nitrogen contents.

Trophic niche comparison of butterfly families

The trophic niche comparisons indicated that the butterfly families strongly differed with regard to the host-plant nitrogen content. On average, lycaenids, independent of the level of myrmecophily, select host plants with higher nitrogen contents in comparison with other butterflies, and myrmecophilous lycaenids attract ants for indirect defence (Pierce *et al.*, 2002). Thus, when caterpillars feed on high-quality host plants, the nutritional content and/or quantity of their reward is higher; consequently, ants will attend these caterpillars in larger numbers, providing increased protection. As suggested by Pierce (1985), a large quantity of high-quality nectar is required to maintain a highly rewarding mutualism and is expected to impose constraints on the food plant choice of the caterpillar.

The Pieridae family also displays preferences for nitrogen-rich host plants in the plant families Brassicaceae
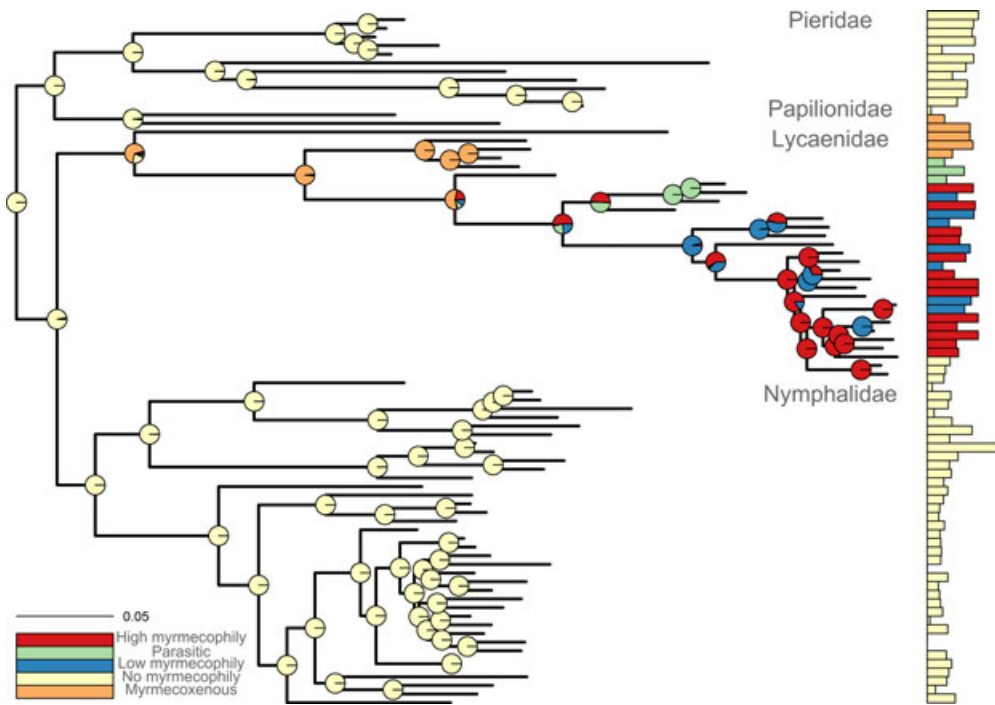
Fig. 2 Phylogenetic tree of the sampled mountain butterfly species either belonging to the nonmyrmecophilous Nymphalidae, Riodinidae (one species only), Pieridae or Papilionidae or belonging to the Lycaenidae. The latter comprise myrmecoxenous, myrmecophilous and ant-parasitic butterflies. The size of the bars represents the average amount of nitrogen in the host plants associated with each butterfly species.
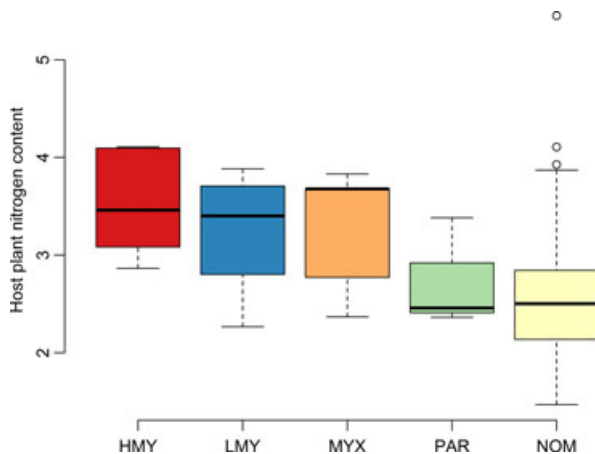

Fig. 3 Boxplot of the average nitrogen content in the food plants of butterfly species either belonging to the nonmyrmecophilous Pieridae, Papilionidae and Nymphalidae (NOM – far right) or belonging to the highly myrmecophilous (HMY), weakly myrmecophilous (LMY), myrmecoxenous (MYX) and ant-parasitic lycaenids (PAR). Most lycaenids (regardless of their degree of myrmecophily) have the same preference for nutrient-rich food plants, whereas the ant-parasitic lycaenid species do not have such a preference because they obtain important quantities of protein once living in the host ants' nest.

(subfamily Pierinae) and Fabaceae (subfamilies Coliadinae and Dismorphiinae). Hence, Pieridae displays a trophic niche highly similar to that of the myrmecoph-ilous Lycaenidae. Caterpillars feeding on host plants containing higher levels of nitrogen generally have increased performance in terms of growth (Barros & Zucoloto, 1999) or survival (Loader & Damman, 1991). Even if not ant-attended, feeding on nitrogen-rich host, plants may still provide significant fitness benefits.

Nymphalidae occupy trophic niches that are much larger compared to other butterfly families, and there is a large heterogeneity in host-plant use across the subfamilies and tribes of nymphalids. For instance, the Satyrinae feed on grasses and sedges, the Nymphalinae on Urticales, Heliconiinae on Violaceae and Melitinae on plants containing iridoid glycosides (Ackery, 1988). The colonization of different host-plant clades is likely to have driven species diversification within this family (Fordyce, 2010). Hence, the larger trophic niche space used locally in the Alps reflects the larger diversity of the host-plant clades colonized by the various lineages of this globally distributed, species-rich butterfly family. Species belonging to the Nymphalidae also have colonized a larger environmental space with regard to other dimensions, including high-elevation environments with more stressful climates (Pellissier *et al.*, in press). One likely explanation that still requires formal testing is that to colonize these environments, Nymphalidae were constrained to associating with host plants that contain a lower level of nitrogen, a characteristic of many plant species at higher elevations (Pellissier *et al.*, in press). As a consequence,
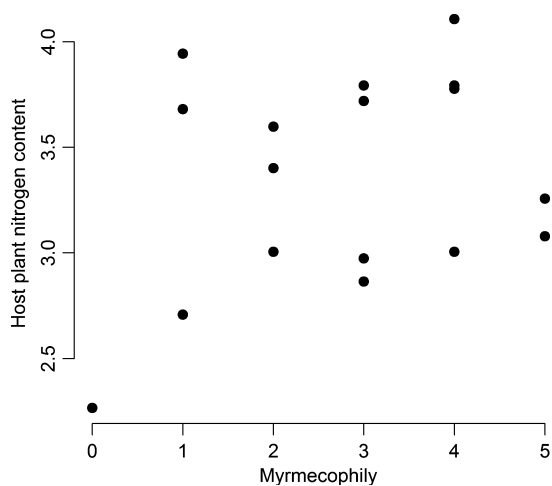
Fig. 4 Relationship between the average host-plant nitrogen content and the level of myrmecophily in Polyommatinae butterflies from the Western Alps. No significant relationship was found.

the trophic niche displayed by this family comprises some nutrient-rich plant species at low elevations but also plant species with a lower nutrient content and tougher leaf structure at high elevations.

In accordance with the predictions, in the absence of other constraints, insect herbivores should be expected to select host plants with the highest nitrogen content. Thus, it is unclear why all butterflies do not feed on nitrogen-rich plants. One explanation is that plants are known to contain a series of toxic secondary metabolites that are also important drivers of host-plant choice (Awmack & Leather, 2002). It may be that Nymphalidae have colonized host plants with lower nitrogen contents because they were unable to overcome the specific defences of those host plants with higher nitrogen contents (e.g. cyanogenic glycosides in Fabaceae). Therefore, host-plant choice results from a trade-off between maximizing nitrogen (and other metabolites, such as P and K) and reducing the intake of digestibility reducers or toxins. Secondly, it is also possible that a reduced competition for nitrogen-poor plants as food increases the favourability of host plants with lower nitrogen contents, as suggested by Janzen (1973); however, we are not aware of any studies that provide evidence for this.

Phylogenetic analysis of myrmecophily levels and host-plant nitrogen content

The OU model accounting for the myrmecophily level was better able to explain the nitrogen-related host-plant choice of butterflies. As with the previous analyses, our results indicated that overall the lycaenids select host plants with higher nitrogen contents compared to other butterflies. Interestingly, the myrmecoxenous species shared similar preferences for those host plants with high foliar nitrogen contents, for example *Lycaena* species

feeding on representatives of the Polygonaceae plant family or *Callophrys rubi* feeding primarily on the Fabaceae family (Fiedler *et al.*, 1993). One possible explanation for this observation is that the colonization of nitrogen-rich host plants in the ancestors of the current myrmecophilous species has served as an exaptation (*sensu* Jay *et al.*, 2011), that is, it has facilitated the initial evolution of dorsal nectar organs and production of nectar exudates. If so, this *a priori* condition, retained through phylogenetic trait conservatism, may explain why many lycaenid species that do not produce costly nectar exudates still prefer nitrogen-rich species as their host plants.

For the ant-parasitic lycaenids, the constraints to feed on nutrient-rich host plants that are imposed by the production of nectar are relaxed because these species feed on ant brood or rely on trophallaxis to obtain high quantities of nitrogen. Parasitic species obtain more than 90% of their biomass during their nonherbivorous ant-parasitic stage (Elmes, 2001). This property allows for the colonization of nutrient-poor plants, especially because the production and delivery of nectar is largely restricted to the short adoption phase and essentially ceases once the individual is adopted into the host ant colony (Wardlaw *et al.*, 2000). As a consequence, the plant nutrient content may not be as important in parasitic species as in herbivorous lycaenids; furthermore, the selective pressures on oviposition plant choice may impose different constraints. The major selective pressure during egg laying might be to select for plants that increase the likelihood of adoption, whereas the nutritive constraints become relaxed (Thomas & Elmes, 2001, Fürst & Nash, 2010).

Host-plant nitrogen content and the strength of myrmecophily

We found no relationship between the strength of myrmecophily and the host-plant nitrogen content in Polyommatinae (Lycaenidae): species having few interactions with ants still feed on nutrient-rich Fabaceae species. This result may indicate that feeding on nitrogen-rich plants should also generally be beneficial for caterpillars that do not produce nectar exudates. Indeed, in addition to finding that the myrmecoxenous species are frequently associated with nitrogen-rich plants, our results showed that several lineages within Polyommatinae have reduced their myrmecophily; however, this was unrelated to a shift away from nutrient-rich plants (except for *Plebejus glandon* and *Celastrina argiolus*). The secondary reduction in myrmecophily in Polyommatinae is likely to be primarily driven by environmental conditions, as suggested by Pellissier *et al.* (in press). Evolutionary conservatism for host-plant affiliation may explain these observed patterns accordingly.

Limitations of the study and future research

One caveat of this study is that we were not able to achieve optimal resolution with regard to the exact diet

of each butterfly species. Therefore, we relied on measuring the 250 most abundant plant species in the study area, hence supposing that the butterflies are likely to rely more on the most abundant and frequently found host-plant species. However, this might not hold true when considering that many species are host specific to some degree, sometimes feeding on rare plants. Additional sources of bias might also be derived from other unmeasured plant-associated traits: for example, some lycaenid caterpillars prefer inflorescences over foliage if available or use the youngest and most tender foliage instead of mature 'fully developed' leaves, which may be more variable in nitrogen content, instead of mature 'fully developed' leaves. Furthermore, phylogenetically conserved classes of plant secondary metabolites can affect herbivore host-plant choice during radiation (Ehrlich & Raven, 1964; Wheat *et al.*, 2007). Finally, due to the amount of work required to measure leaf properties for a large number of plant species, we only investigated this pattern for a given number of species distributed in this alpine region. Complementary studies in other regions could help to evaluate the generality of the pattern revealed in this study.

Conclusions

We used complementary approaches to investigate the role of the host-plant nitrogen content in driving myrmecophily levels across different phylogenetic scales. Our data were consistent with the evolutionary shifts to high-nutrient host plants serving as exaptation for the evolution of myrmecophily in lycaenids. Butterfly species in the nonmyrmecophilous family Pieridae also prefer nitrogen-rich host plants. Overall, nitrogen is an important component of the caterpillar diet for both myrmecophilous and nonmyrmecophilous species as it can enhance both larval fitness and survival. Yet, when nitrogen can be obtained through alternative means, as in socially parasitic lycaenid species feeding on ant brood, the selective pressure for maintaining the use of nutrient-rich host plants is relaxed, allowing for the colonization of alternative host-plant families. Finally, constraints apart from the host plants (e.g. the production of chemical compounds) may limit the feeding by caterpillar species, such as those in the Nymphalidae family, on host plants with the highest nitrogen content.

Acknowledgments

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 List of host plant species known for the butterfly fauna in the study area.

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