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Performance of leaf beetle larvae on sympatric host and non-host plants

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Abstract

Studies asking the ability of insects to utilize novel host plants often use novel hosts that are allopatric with the insect population under investigation. However, since the outcomes of species interactions are often site-specific, such studies cannot tell us whether a plant would actually be used by a given insect population if the plant grew sympatrically with it. We therefore performed a quantitative genetics experiment to analyse the performance of larvae of the leaf beetle *Oreina elongata* Suffrian (Coleoptera: Chrysomelidae, Chrysomelinae) on two host and three non-host plants, collected from a site where insects and plants co-occur in the Western Alps. When raised on the non-host *Petasites albus* (L.), larvae were able to survive equally well as on the two hosts, *Adenostyles alliariae* (Gouan) and *Cirsium spinosissimum* (L.), whereas they did not survive on the two other non-hosts, *Peucedanum ostruthium* (L.) and *Rumex alpinus* L. On *P. albus*, growth rate was slightly lower and development time slightly longer than on the two hosts. We found a genotype by environment interaction only for growth rate but not for development time and survival. However, the shape of the reaction norms of growth rates suggests that it is unlikely that selection could favour the inclusion of *P. albus* into the host range of the study population.

Introduction

The vast majority of phytophagous insects are specialised in their use of only few host plant species, mainly belonging to the same family. In this context, the physiology of insects and plants is obviously a very important selective factor in shaping host plant use since phytophagous insects must be adapted to digest plant nutrients and to deal with plant defensive secondary compounds. However, several examples are known of insect species that use a range of host plants that is actually narrower than the range of plants on which the physiology alone would allow larvae to develop and survive (e.g., Wiklund, 1975; Kibota & Courtney, 1991; Futuyma et al., 1994, 1995; more references in Fox & Lalonde, 1993). On one hand, ecological factors, such as natural enemies, competition or meteorological adversities may concur to restrict an insect's host range (Futuyma & Peterson, 1985; Denno et al., 1990; Jaenike, 1990). On the other hand, ovipositing females may not accept, or may not be attracted to, the chemical or visual stimuli of some of the plants on which their larvae might perform well (Jaenike, 1985; Fox & Lalonde, 1993). When larvae are preadapted to grow and survive on a wider number of plants than those actually used, then a species' host range might expand if females oviposited by mistake on a potential host not normally utilised (Feeny, 1991; Jaenike & Papaj, 1992; Larsson & Ekbom, 1995). Moreover, if adults showed fidelity to the novel host on which they developed as larvae, then a new host-specific insect race could potentially evolve (Wood et al., 1999). Oviposition mistakes are well documented for lepidopterans and gall makers, the adults of which are very mobile and fly around searching for plant patches for suitable hosts (references in Larsson & Ekbom, 1995). In addition, mistakes are more likely if females that carry high egg loads end up on novel plants that are chemically similar to the normal hosts (Jaenike, 1990).

Studies measuring insect survival and growth on novel hosts are often performed using either labora-

tory insect cultures or novel hosts that are allopatric with the studied insect population (e.g., Kibota & Courtney, 1991; Futuyma et al., 1994, 1995; Thompson, 1996; Gratton & Welter, 1998; Lazarevic et al., 1998; Panizzi & Oliveira, 1998). Although studies have shown that insects can be preadapted to live on a novel host, the failure to explicitly test sympatric insects and plants complicates any inference concerning whether these insects would actually use the novel host if it co-occurred with the normal host in nature. This is an important point since it is now recognized that the outcome of the interaction between two species is often site-specific (Thompson, 1994).

It has been postulated that an insect's physiological specialisation on one or few host plants should be favoured by the existence of trade-offs of larval performance among hosts (see Rausher, 1988, 1992; Via, 1990; Jaenike, 1990). Such trade-offs should be linked to negative genetic correlations of larval fitness traits across hosts, favouring the evolution of host-specialised genotypes that could theoretically lead to speciation (see Via, 1990; Jaenike, 1990). However, negative across-host genetic correlations of larval performance traits have rarely been found (e.g., recently Ueno et al., 1997; Keese, 1998; Lazarevic et al., 1998; discussed in, among others, Rausher, 1988, 1992; Jaenike, 1990; Via, 1990; Thompson, 1994; Joshi & Thompson, 1995; Fry, 1996). Recently, it has been proposed that negative genetic correlations may not be necessary for host specialisation to evolve, but that a significant statistical interaction of larval performance traits between insect genotypes and host plant could suffice (Fry, 1996).

In this study, we compared the larval performances of different genotypes (families) of the specialised leaf beetle *Oreina elongata* Suffrian (Coleoptera: Chrysomelidae, Chrysomelinae) on two of its normal hosts and three non-host plant species. All the beetle genotypes and plants came from the same site. One of the three non-host plants is a host to an *Oreina* species that is closely related to *O. elongata*, the second one is host to a phylogenetically more distant *Oreina* and the third one is not used by any beetle in this genus. Our goals were: (1) to test whether the non-use of the three sympatric non-hosts is due to an inability of *O. elongata* larvae to survive and grow on these plants and (2) to infer whether there is a potential for host range evolution through genotype by host interactions on performance traits.

Materials and methods

Study organisms. Oreina elongata feeds and oviposits on two host plants that belong to different tribes of the family Asteraceae. *Adenostyles alliariae* (Gouan) is characterised by large (up to about 300 mm broad) heart-shaped, smooth leaves that contain pyrrolizidine alkaloids (PAs), which can be sequestered by *O. elongata* larvae and adults and used as chemical defences against natural enemies (Dobler & Rowell-Rahier, 1994a). The second host plant, *Cirsium spinosissimum* (L.), has strongly dentate (up to about 200 mm long and 60 mm broad) hairy and spiny leaves and does not contain any secondary metabolites that could be used by the beetle for chemical defence (Dobler & Rowell-Rahier, 1994a). However, those *O. elongata* that live in sites where *A. alliariae* is absent can rely on an endogenous synthesis of cardenolides as defensive compounds (Dobler & Rowell-Rahier, 1994a). Some populations of *O. elongata* inhabit places where only one of the two host plants is present and others live in the presence of both plants (Dobler & Rowell-Rahier, 1994b; Dobler et al., 1996; Pasteels et al., 1996).

All three of the non-host plants tested grow on the site of the population studied, in the Western Alps (see below). Patches of each of the non-hosts can be found between zero and only few meters away from colonised patches of both normal hosts. We tested the following non-hosts: (1) *Petasites albus* (L.) (Asteraceae) does not contain any PAs (Pasteels et al., 1996) and has large round and smooth leaves that very much resemble the leaves of *A. alliariae*. In other locations, *P. albus* is used as a host by *O. speciosissima*, a species that is phylogenetically very close to *O. elongata* (Dobler et al., 1996; Hsiao & Pasteels, 1999). (2) *Peucedanum ostruthium* (L.) (Apiaceae), a species which is host of the phylogenetically more distant *O. gloriosa* (Dobler et al., 1996; Hsiao & Pasteels, 1999). This is a plant without PAs but its leaves contain furanocoumarins. In our study area, *O. gloriosa* is present at very low densities (P. Ballabeni & D. Conconi, personal observations). (3) *Rumex alpinus* L. (Polygonaceae) is not a host for any *Oreina* species but is abundant in our study site where it serves as host for another chrysomelid beetle, *Gastrophysa viridula* (Chrysomelinae) (De Geer).

Study population. The population studied is located in the western Alps, on the pass of the Petit Saint-Bernard, at the border between the French region of Savoie and the Italian region of Vallée d'Aoste. The

pass lies at 2188 meters elevation and is therefore above the tree line. Winters are severe and summers short. *Oreina elongata* is mainly active in July and August. Adults emerge from overwintering in the ground and start mating between the end of June and mid July, before the snow has completely melted. Eggs are laid until the beginning of August when the adults disappear either because they die or because they hide, probably in their overwintering sites. A mark-recapture study suggests that at least one fifth of the adults undergo two consecutive reproductive seasons (D. Conconi, unpubl.).

At the study site, *O. elongata* shows a strong oviposition preference for *C. spinosissimum*, probably because its eggs survive better on this plant than on *A. alliariae* (P. Ballabeni, D. Conconi, S. Gateff & M. Rahier, unpubl.). However, *O. elongata* only oviposits on those *C. spinosissimum* plants that grow in very close proximity to *A. alliariae*, this latter being the plant that allows faster larval growth under laboratory conditions (Ballabeni & Rahier, 2000). In the laboratory, larvae have the highest survival when they feed on a mixture of both plants. Moreover, at the field site, high numbers of larvae move from *C. spinosissimum* to *A. alliariae* during the season (P. Ballabeni, D. Conconi, S. Gateff & M. Rahier, unpubl.; Ballabeni & Rahier, 2000).

Experimental design. We used a sib design to test the effects of diet and insect genotype on larval performance. We produced ten larval families from gravid females we collected in the field in July 1997. The females were collected from large plant patches, containing both host species. Females were individually kept in transparent, round plastic boxes of 90 mm diameter by 50 mm height where they were allowed to lay eggs. To provide necessary humidity, the bottom of each box was covered with a moistened chalk layer, between 5 and 10 mm thick. The chalk was covered with a round filter paper of the same diameter as the box. We distributed the boxes randomly on a shelf in the experimental room. We fed females simultaneously with both *A. alliariae* and *C. spinosissimum* collected from the field site and renewed food every three days. Both the production of the experimental families and the experiment were performed in a building at the field site. The experimental room was not heated and the temperature fluctuated between 7 and 17 °C, values that lie within the range of natural daily temperature fluctuations in the field. Realistic temperature fluctuations can be very important for experiments involving life-history parameters (Brakefield & Mazzotta, 1995).

Once eggs were laid, they were transferred to Petri-dishes of 60 mm diameter containing a moistened chalk floor layered with a filter paper. Each Petri-dish contained between one and five eggs. When the first egg inside a Petri-dish hatched, the other eggs were discarded and the eclosed larva was used for the experiment. Thus, each larva was reared individually inside a Petri-dish. For every family, ten larvae were assigned to the *A. alliariae* diet, ten to *C. spinosissimum*, five to *P. albus*, five to *P. ostruthium* and five to *R. alpinus*. These differences in family size were due to the fact that the *A. alliariae* and the *C. spinosissimum* diet levels were also part of another, larger experiment and that available space and time did not allow for an equally large family size for the five plants (Ballabeni & Rahier, 2000). Petri dishes were randomly distributed on shelves in the same room in which their mothers had laid eggs. Larvae were fed *ad libitum* according to the diet they were randomly assigned, whereby the food was changed every two days after being collected from the field on the same day.

We checked the larvae daily for mortality and developmental stage. We weighed the larvae on the hatching day and one day after their third moult, when the experiment was stopped. The third moult is the last one *O. elongata* undergoes before pupation. We ended the experiment at the third moult because it is not possible to make *O. elongata* successfully pupate under laboratory conditions. We did not weigh the larvae on the exact day of the third moult because moulting is accompanied by water losses.

For each larva, we registered the following performance characters: survival (surviving or not to the third moult), growth rate (mg of weight increase per day from hatching to third moult) and development time (number of days from hatching to third moult).

Statistical analyses. The effects of the genotype (family), environment (diet) and their interaction on each performance character were tested with mixed-model analyses of variance (ANOVA). The genotype and the interaction were considered random effects and the environment a fixed effect. Given that the experimental design was unbalanced, we used the GLM procedure (general linear models) of the SAS statistical package, with type III sums of squares (SAS Institute, 1989; Potvin, 1993). For the analyses, the binary survival data were coded as 0 (larva died before third moult) or 1 (larva survived to third moult), fol-

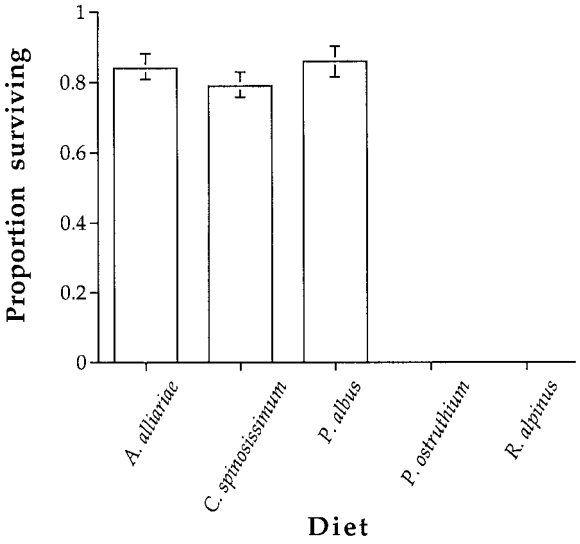

Figure 1. Effect of diet on the proportion of larvae that survived until third the moult. Error bars show standard errors.

Table 1. ANOVA for larval survival to third moult

Source of variation	df	MS	F-value	P
Family	9	0.1957	2.02	0.060
Diet	4	12.1761	125.91	<0.001
Family × diet	36	0.0967	0.98	0.511
Error	300	0.0990		

lowing standard practice (Falconer, 1989; Roff, 1997). Growth rates and development times were transformed by their natural logarithm to meet model assumptions (Sokal & Rohlf, 1995). The coded survival data remained untransformed (Roff, 1997).

Results

Diet had a highly significant influence on larval survival between eclosion and third moult (Table 1). Larvae survived equally well on the two host plants *A. alliariae* and *C. spinosissimum* and on the non-host plant *P. albus* but could not survive on the other two non-hosts *P. ostruthium* and *R. alpinus* (Figure 1). The latter two plants were not eaten by the larvae, which died within a few days after eclosion. The interaction between family and diet was not significant, implying that there was no genetic variation for phenotypic plasticity on survival (Table 1).

Growth rate could be analysed only for larvae raised on *A. alliariae*, *C. spinosissimum* and *P. albus*, given the 100% mortality of the larvae reared on the two other plants. The interaction between family and diet significantly affected growth rate (Table 2). Hence, the reaction norms for growth rate significantly crossed, i.e., the insect families had different performance ranks on the different diets. Once again, diet had a strongly significant effect (Table 2). The larvae reared on *A. alliariae* grew fastest, the ones raised on *C. spinosissimum* at an intermediate rate, and those raised on *P. albus* slowest (Figure 2).

Diet had a significant effect on larval development time (Table 3). Development time was shortest for larvae reared on *A. alliariae*, intermediate for larvae fed on *C. spinosissimum* and longest for larvae fed on *P. albus* (Figure 3). As was the case for survival, no significant interaction was detected between diet and development time (Table 3).

Table 2. ANOVA for larval growth rate to third moult. Data were ln-transformed for analysis

Source of variation	df	MS	F-value	P
Family	9	0.1342	1.52	0.211
Diet	2	3.0319	34.13	<0.001
Family × diet	18	0.0910	2.02	0.011
Error	173	0.0451		

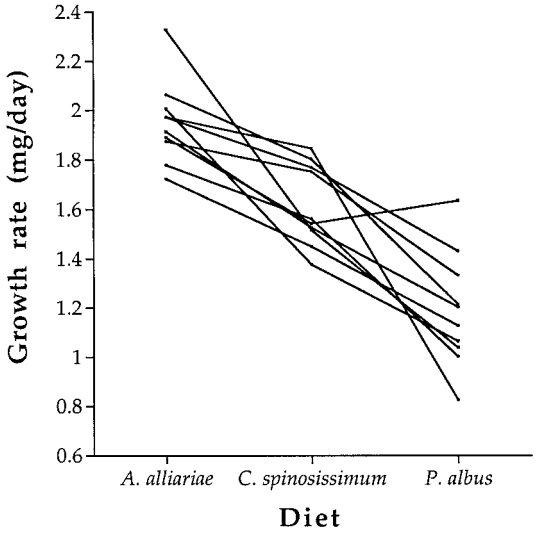

Figure 2. Family-diet interaction for larval growth rate. Each line connects the mean values for sibs fed different diets in a split-family design and therefore represents the family's reaction norm. Means were calculated on ln-transormed values and then transformed back to mg/d units.

Table 3. ANOVA for larval development time to third moult. Data were ln-transformed for analysis

Family	9	0.0138	1.36	0.267
Diet	2	0.1635	16.19	<0.001
Family × diet	18	0.0101	1.00	0.461
Error	176	0.0101		

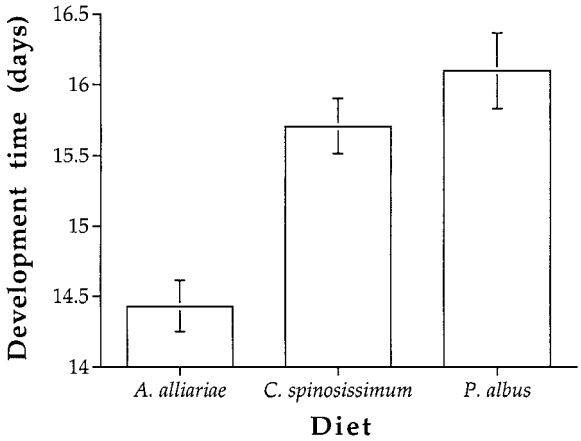

Figure 3. Effect of diet on the time larvae needed to develop between eclosion and third moult. Error bars show standard errors. Means and standard errors were calculated on the ln-transformed values and then transformed back to day-units.

Discussion

Our results show that larvae of *O. elongata* from Petit Saint-Bernard are able to survive and grow on *P. albus*. However, this plant, which is abundant at our field site and elsewhere, is never used by the beetle in nature (Dobler & Rowell-Rahier, 1994b; Dobler et al., 1996; Pasteels et al., 1996). In contrast, larvae were not able to feed and survive on *P. ostruthium* or *R. alpinus*.

Why is *P. albus* not used as a host plant by *O. elongata*? We can seek, on one side, proximate explanations related to the behaviour of the ovipositing females and, on the other side, ultimate explanations related to natural selection acting on eggs or larvae (see references in Fox & Lalonde, 1993). These two classes of explanations are not mutually exclusive. Among the first group of explanations, plant apparency, phenology, abundance, and reliability as well as the sensorial interactions between insect and plant can be mentioned (Fox & Lalonde, 1993). We can exclude the first four reasons, because *P. albus* is not less apparent than the two normal hosts of *O. elongata*, and has a comparable abundance, reliability, and phenology to the two normal hosts. However, we do not know whether the chemical signals emitted by *P. albus* fail to attract ovipositing females of *O. elongata*.

It is well established that selection through natural enemies can oppose the use of suitable plants (e.g., Denno et al., 1990; Feder, 1995; Keese, 1997; Rank et al., 1998). Thus, *P. albus* may not be used by *O. elongata* in the wild because it does not provide either the advantages of sequestrable defensive compounds or those of a protective leaf anatomy. In the population studied, the ovipositing beetles seek a very close spatial proximity between *A. alliariae* and *C. spinosissimum*, whereby the first plant allows faster larval growth and provides sequestrable PAs and the second one allows a higher egg survival (Ballabeni & Rahier, 2000; P. Ballabeni, D. Conconi, S. Gateff & M. Rahier, unpubl.). If *O. elongata* maximises its fitness by ovipositing on the plant which maximises egg survival, close to the plant that maximises larval performances and provides chemical defenses, then the use of *P. albus* would be maladaptive. It would be interesting to know whether *P. albus* would be used as a host if *A. alliariae* or *C. spinosissimum* or both plants disappeared from the site. But we know of no *O. elongata* population that uses *P. albus*, independently of the presence or absence of either *A. alliariae* or *C. spinosissimum*. It should be kept in mind, however, that in our study we could not follow larval development until pupation. We cannot exclude that, in nature, *P. albus* is avoided because it might lack some nutrient that is important to complete development beyond the pupal stage.

An important experimental component of the present study was the sympatry between the beetle and *P. albus*. Several studies showing that insect larvae were able to survive and grow on non-host plants were performed using plants and insects from allopatric populations (e.g., several of the non-hosts in Wiklund, 1975; Kibota & Courtney, 1991; Futuyma et al., 1994; Thompson, 1996). Explicitly stating the geographic origin of both the plants and the insects tested in an experiment is very important since the outcomes of the interaction between a given insect species and a given plant species can vary geographically (Thompson, 1994). Relatively simple plant genetic mechanisms can suffice to cause such geographic variations (e.g. Nielsen, 1996; Linhart & Thompson, 1999). For instance, the flea beetle *Phyllotreta nemorum* uses three species of *Barbarea* (Brassicaceae) as hosts in Denmark (Nielsen, 1996). Only certain populations of the most common of the three plants, *B. vulgaris*,

are used as hosts by the beetle since most *B. vulgaris* populations are toxic to *P. nemorum*. However, some beetle populations live on *B. vulgaris* that are toxic to beetles from other sites. A small number of insect genes is responsible for this pattern of resistance and susceptibility (Nielsen, 1996). Thus, the fact that an allopatric plant is an accepted host under experimental conditions does not necessarily mean that it would be used by a sympatric insect population in nature.

Phylogenetic considerations may help explaining why *O. elongata* is able to survive on *P. albus* but not on *P. ostruthium*. In several locations, *P. albus* is host to *O. speciosissima*, which, together with *O. cacaliae*, is the species that is phylogenetically closest to *O. elongata* (Dobler et al., 1996; Hsiao & Pasteels, 1999). *Oreina elongata* or its immediate ancestor might therefore have used *P. albus* in the past, which could explain why larvae of *O. elongata* are able to survive on *P. albus*. In contrast, *P. ostruthium* contains furanocoumarins, defensive chemicals that are photoactive and toxic to insects (Berenbaum, 1978). Within the genus *Oreina*, only *O. gloriosa* has evolved strict specialisation on *P. ostruthium*. Larvae of this beetle are active at night, a possible adaptation to circumvent the plant chemical defenses (L. Nessi, unpubl.). *Oreina gloriosa* belongs, together with a few other species that accept *P. ostruthium* as a food plant, to a clade that is phylogenetically distant from *O. elongata* (Dobler et al., 1996; Hsiao & Pasteels, 1999). This clade has evolved feeding on *P. ostruthium* after its separation from the clade that has lead to *O. elongata* (Dobler et al., 1996). Since *R. alpinus* belongs to a family that is never used as a host by the genus *Oreina*, it is not surprising that it was not accepted in our experiment.

Growth rate was the only performance trait for which we found an interaction between genotype (family) and environment (diet). This interaction suggests that there is the potential for the evolution of host-specialised genotypes within the study population (Via, 1990; Fry, 1996). A look at the reaction norms of growth rate shows that each family performed better on *A. alliariae* than on *P. albus* and that only one family performed better on *P. albus* than on *C. spinosissimum*. Thus, even if we consider growth rate alone, it seems that there is only little room for selection to favour specialisation on *P. albus*, in spite of the genotype by environment interaction.

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