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A quantitative genetic analysis of leaf beetle larval performance on two natural hosts: including a mixed diet

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Abstract

Published quantitative genetic studies of larval performance on different host plants have always compared performance on one host species or genotype vs. performance on another species or genotype. The fact that some insects may feed on more than one plant species during their development has been neglected. We executed a quantitative genetic analysis of performance with larvae of the leaf beetle *Oreina elongata*, raised on each of two sympatric host plants or on a mixture of them. Growth rate was higher for larvae feeding on *Adenostyles alliariae*, intermediate on the mixed diet and lowest on *Cirsium spinosissimum*. Development time was shortest on *A. alliariae*, intermediate on mixed diet and longest on *C. spinosissimum*. Survival was higher on the mixed diet than on both pure hosts. Genetic variation was present for all three performance traits but a genotype by host interaction was found only for growth rate. However, the reaction norms for growth rate are unlikely to evolve towards an optimal shape because of a lack of heritability of growth rate in each single environment. We found no negative genetic correlations for performance traits among hosts. Therefore, our results do not support a hypothesis predicting the existence of between-host trade-offs in performance when both hosts are sympatric with an insect population. We conclude that the evolution of host specialized genotypes is unlikely in the study population.

Keywords:

genetic correlation; larval performance; mixed diet; *Oreina*; reaction norm; trade-off.

Introduction

Most phytophagous insect species are known to be specialized in their use of very few species of host plants for oviposition and feeding, mostly belonging to the same family (Strong *et al.*, 1984; Jaenike, 1990). The existence of between-plant trade-offs for larval performance, due to antagonistic pleiotropic effects of alleles for host use, has been hypothesized to explain this pattern (see Rausher, 1984; Diehl & Bush, 1989; Jaenike, 1990; Joshi & Thompson, 1995; Fry, 1996). Thus, an evolutionary change in allele frequencies that causes an improved fitness on one host would cause a lower fitness on the other hosts and negative genetic correlations for insect larval performance on different plants should be found (Rausher, 1984; Diehl & Bush, 1989). In addition to favouring host specialization, between-plant negative genetic correlations of larval performance could theoretically also cause genetic divergence in host use within insect species. Such a process might lead phytophagous insects to the formation of host-specialized races and eventually to speciation (Diehl & Bush, 1989).

Quantitative genetic studies of insect performance on different host plants have neglected mixed diets. We do not know of any published studies which have tried to infer between-host genetic correlations or reaction norms that included a dietary mixing of hosts as a third environment. Typically, studies have compared performances of larvae feeding purely on each of two host plants, in spite of the fact that several insect species have larvae that frequently switch between host plants during their development. Frequent host switching during feeding have especially been found in several Lepidoptera and Orthoptera larvae (see Bernays & Bright, 1993;

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references in Schoonhoven *et al*., 1998) but also during the development of Hemiptera (Brodbeck *et al*., 1995). Dietary mixing has often been shown to enhance larval performance traits, like survival or fecundity, in butterflies and grasshoppers (Schoonhoven *et al*., 1998). Mixed diets may generally provide more balanced supplies of nutrients and/or dilute plant toxins (Freeland & Janzen, 1974; Pulliam, 1975; Rapport, 1980; Slansky, 1992; Bernays & Bright, 1993; Raubenheimer & Simpson, 1993). However, there are few, if any, studies of the effects of dietary mixing on the larval performance of oligophagous species. On the other hand, it is obviously the oligophagous insects that are the objects of the studies on the evolution of host specialization. Insects that feed on more than one host species during their development would perceive their hosts as a fine-grained environment, whereas insects that do not move between host species would perceive their environment as coarse-grained (Via, 1994). A fined-grained environment is less likely to maintain genetic polymorphism within a population than a coarse-grained one (Hedrick *et al*., 1976) and may produce patterns of reaction norms and genetic covariation in performance traits that differ from the patterns produced by a coarse-grained environment (Via, 1994). In other words, whether or not a mixed diet is used is likely to influence the evolutionary trajectory of the insect–plant relationship within a population.

Most of the studies published have not found any evidence for performance trade-offs between hosts since the genetic correlations found were either zero or positive (e.g. recently, Ueno *et al*., 1997; Keese, 1998; Lazarevic *et al*., 1998; see discussions in, among others, Rausher, 1988; Jaenike, 1990; Via, 1990; Thompson, 1994; Joshi & Thompson, 1995; Fry, 1996). Methodological, ecological or genetic explanations have been put forward to explain the lack of negative genetic correlations (Rausher, 1988; Jaenike, 1990; Fry, 1993; Joshi & Thompson, 1995).

According to the conventional wisdom, an insect population that has adapted to two sympatric host plants may be expected to lack between-host trade-offs of larval performance traits (Via & Lande, 1985; Rausher, 1988). However, an alternative hypothesis suggests that the age of a given insect–plant relationship may also play a role (Joshi & Thompson, 1995). Trade-offs for larval performance should be found only between normal hosts but not if one compares a normal vs. a novel host. For normal hosts, the alleles that maximize performance on one or the other host, or on both simultaneously, should be fixed once genetic equilibrium has been reached, whereas the alleles that have negative between-host pleiotropic effects would remain variable. Trade-offs would therefore be apparent. In a test of a normal vs. a novel host, alleles with between-host positive pleiotropic effects would not have gone to fixation yet and would reduce or mask the trade-offs due to alleles with negative pleiotropic effects (Joshi & Thompson, 1995).

Finally, the necessity of between-host performance trade-offs for the evolution of host specialization to occur has recently been challenged (Fry, 1996). A mathematical model showed that a significant genotype by host interaction could be sufficient to allow the evolution of host specialization, even in the absence of negative genetic correlations. This result may be very important for the study of the evolution of host specialization because several studies have found genotype by host interactions in the absence of negative genetic correlations (e.g. recently, Thompson, 1996; Ueno *et al*., 1997; Keese, 1998; Lazarevic *et al*., 1998).

In the present study we used a quantitative genetics framework to investigate the larval performance of a specialized leaf beetle population when reared on each of its two sympatric host plants and on a mixture of both plants. The study population experiences its two hosts as a relatively fine-grained environment (see below). Our aims were to: (1) find out whether the larvae perform better on the plant that is known to be preferred for the oviposition, as would be expected if larval performance alone selected for oviposition preference; (2) to test whether the reaction norms and genetic correlations correspond to the prediction of no trade-offs between normal hosts or to the prediction of existing trade-offs between normal hosts; (3) to use the information given by point 2 to make predictions about the evolution of host use when hosts are perceived as a fine-grained environment.

Materials and methods

Study organisms

Oreina elongata Suffrian (Coleoptera: Chrysomelidae) is specialized in the use of only two host plants that belong to family Asteraceae. The beetle oviposits and feeds on *Adenostyles alliariae* (Gouan), a plant that produces pyrrolizidine alkaloids (PAs) which are sequestered by both the adults and the larvae and used as chemical defences, and on *Cirsium spinosissimum* (L.) (Dobler & Rowell-Rahier, 1994). *C. spinosissimum* does not provide the beetle with any sequestrable defensive chemical compounds but *O. elongata* of populations that live in places where only this plant is present are able to rely on self-synthesized cardenolides for their defence (Dobler & Rowell-Rahier, 1994). Unlike *A. alliariae*, *C. spinosissimum* has very spiny and hairy, dentate leaves that may give some degree of mechanical protection to *O. elongata* eggs or larvae. PAs seem to protect the beetles more efficiently than cardenolides, at least against generalist avian predators (Rowell-Rahier *et al*., 1995). We know *O. elongata* populations that live in places in which only one or the other host plant is present and others that live in the presence of both hosts (Dobler & Rowell-Rahier, 1994; Dobler *et al*., 1996; Pasteels *et al*., 1996). Our study is about an *O. elongata* population that is sympatric with

both host plants and uses both for oviposition and feeding.

Study site and population

We investigated the *O. elongata* population of the Petit Saint-Bernard Pass, which lies in the Western Alps, at the border between the regions of Savoie, France, and Vallée d'Aoste, Italy. The pass is located above the tree line at 2188 m elevation. *A. alliariae* and *C. spinosissimum* are present in patches of various sizes, which include either one or the other plant species or both. The area is characterized by severe winters and short summers. Adults of *O. elongata* start their reproductive season when the snow has almost completely melted, usually at the end of June but in some years not before mid July. Eggs are laid through the month of July until the first half of August. Larvae feed on both host plants until September.

A field investigation showed that, in this population, *C. spinosissimum* is much preferred over *A. alliariae* for oviposition and that those plants of *C. spinosissimum* that grow next to *A. alliariae* are much preferred over the plants of *C. spinosissimum* that grow a few metres away from the other plant (P. Ballabeni *et al*., unpublished manuscript). Larval feeding on a mixture of both host species should be possible in this population, since larvae are able to move between adjacent hosts within the same day (P. Ballabeni *et al*., unpublished data). Furthermore, larvae move from *C. spinosissimum*, the plant on which they hatch, to *A. alliariae* during their development. The two host plants represent therefore a fine-grained environment for larvae of *O. elongata*.

Experimental design and procedure

We performed this experiment at the Petit Saint-Bernard, in a building located 500 m from the study population. The experiment was organized as a family design with 20 families of 30 larvae each. A half-sib design with each male mated to several virgin females would be ideal for a quantitative genetics study but *O. elongata* cannot be bred in the laboratory. For this reason, we obtained the larval families from field-collected gravid females. Ten larvae of each family were tested for performance on *A. alliariae*, 10 on *C. spinosissimum* and 10 on a mixed, *A. alliariae* + *C. spinosissimum*, diet.

We collected gravid females of *O. elongata* in the field on 8 July 1997 to produce the full sib families. Females were recognized as gravid through their swollen abdomen. Twenty-one females were randomly collected from *A. alliariae* and 20 randomly collected from *C. spinosissimum* and individually kept in round, transparent plastic boxes, 90 mm diameter and 50 mm in height, where they were allowed to lay their eggs. Boxes were randomly positioned on a shelf. Each box's bottom was covered by a 10-mm-thick, moistened chalk layer which was covered with one round filter paper of the same diameter as the box. Each female was simultaneously given both *A. alliariae* and *C. spinosissimum* as food during the egg laying period. Food plants were freshly collected in the study population's field site and renewed every 3 days.

The egg production took place at room temperature. Temperatures fluctuated between 7 and 17 °C, a difference which lies within the limits of the natural temperature range. Realistic temperature fluctuations can be important for the study of life history traits (Brakefield & Mazzotta, 1995). The length of the daily photoperiod varied following the natural seasonal changes since the experiment was performed in a room with windows.

The first 10 *A. alliariae* collected and the first 10 *C. spinosissimum* collected females which laid enough eggs were used to produce the 20 experimental families. Oviposition was checked daily and each newly hatched larva was transferred into an individual Petri dish with a moistened chalk bottom covered with a filter paper. Larvae were randomly assigned to one of the three diet levels, according to the experimental design given above. During the whole experiment, we fed the larvae *ad libitum* with leaves collected on the same day in the field. We stress that both the insects tested and their food plants came from the same site. This may be important if some degree of adaptation by *O. elongata* to its local food plants is present. We changed the food every 2 days. The larvae assigned to the mixed diet food level were alternately fed with one plant species after the other over the entire length of the experiment, whereby the two plants were alternated every 2 days. In this way we avoided the possibility of the larvae choosing between food plants. We also observed that each of these larvae actually ate both plants during the whole experiment. To control for environmental effects, Petri dishes occupied random positions on shelves in the laboratory. Temperature and light conditions were the same as for the egg laying females.

Larvae were checked daily for mortality and developmental stage. We weighed each larva on the hatching day and one day after third moult and we noted the number of days from hatching to third moult. We avoided weighing the larvae on the exact day of third moult because moulting is accompanied by important and inconsistent water losses which make comparisons impossible, whereas weight differences are comparable one day after moult. We ended the experiment at the third moult, i.e. at the beginning of the last instar, because it is not possible to make *O. elongata* pupate in the laboratory.

We calculated or recorded the following performance parameters for each individual larva: daily growth rate (mg weight increase per day between hatching and 1 day after third moult), development time (number of days from hatching to third moult) and survival (surviving until third moult or not).

Data analysis

Since we were not able to control for larval fatherships, we had to analyse our data under the assumption that we were working with full sib families. This implies that our estimates of heritabilities and genetic correlations may be inflated by dominance and maternal effects rather than being only due to additive genetic effects (Falconer, 1989).

We performed a set of analyses to test the effects of (1) the plant species from which the mothers of the larvae were collected (mother origin), (2) genotype (family), (3) host (diet) and (4) genotype–host interaction on growth rate, development time and survival. Each performance character was analysed with a nested, mixed-model analysis of variance (ANOVAS), in which family was considered as a random effect and diet as a fixed one, and family was nested within mother origin. We used the Scheffé model of ANOVA in which the family mean square was divided by the error mean square (Fry, 1992). In such an analysis, the *F*-test for the family effect is a test of the genetic variability of a trait over all environments (Fry, 1992). We think this is biologically meaningful because, in the study population, *O. elongata* larvae move from *C. spinosissimum* to *A. alliariae* during their development, therefore using both hosts (P. Ballabeni *et al*., unpublished manuscript). Survival data were coded as 0 (larva died before 3rd moult) or 1 (larva was alive at 3rd moult), following the standard quantitative genetics procedures for threshold characters (Falconer, 1989; Roff & Simons, 1997; Roff, 1997). We used type III sum of squares which tolerates unbalanced sample sizes.

Growth rates and development times were ln-transformed before the ANOVAS, to meet the assumptions of homogeneity of variances (Sokal & Rohlf, 1995), whereas coded survival data were not transformed (Roff & Simons, 1997).

Genetic correlations across all possible pairs of diets for each performance character were estimated through the Pearson product-moment correlation of family means (Via, 1984; Roff, 1997). This technique is widely used to calculate genetic correlations (e.g. Via, 1984; Carrière & Roitberg, 1995; Campbell, 1997; Sgrò & Hoffman, 1998) but it is likely to overestimate them (Fry, 1992; Roff, 1997). Since the probability of finding a false significant correlation (type I error) increases with the number of tests that are simultaneously performed, we corrected the *P*-values of the genetic correlations using a sequential Bonferroni technique (Rice, 1989). For the genetic correlations, the family means for growth rates and development times were calculated on ln-transformed data, whereas the data for survival were the untransformed proportions of individuals alive at 3rd moult in each family (Sokal & Rohlf, 1995).

Broad sense heritabilities of the three performance traits on each diet were calculated following the standard formulae based on one-way ANOVAS performed separately for each diet (Falconer, 1989; Roff, 1997), whereby survival was treated as a threshold character and coded 0 or 1, as explained above (Roff, 1997). Again, growth rates and development times were ln-transformed before the ANOVAS whereas the coded survival data were not. We checked whether the heritabilities were statistically different from zero with *t*-tests based on standard errors calculated using the formulae given by Roff (1997).

Mean values of the performance traits are given ± their standard errors. Statistics were calculated with the JMP software (SAS, 1989).

Results

Effects of diet on performance and norms of reaction

Both larval genotype and host influenced the growth rate, as shown by the significant family and diet effects (Table 1). Overall, mean growth rates were higher on *A. alliariae* (1.92 ± 0.030 mg day^{-1}) than on the mixed diet (1.66 ± 0.029 mg day^{-1}) or on *C. spinosissimum* (1.61 ± 0.031 mg day^{-1}) (Fig. 1). The significant interaction between family and diet (Table 1) shows genetic variability for the reaction norms (Fig. 1). Different genotypes thus ranked differently on the different hosts (Fig. 1). The plant species from which we collected the mothers of the experimental larvae did not influence growth rates (Table 1).

For development time, we found genetic variation (significant family effect) and a significant effect of diet (Table 2). Development time was shortest on *A. alliariae* (16.55 ± 0.137 days), intermediate on the mixed diet (17.30 ± 0.134 days) and longest on *C. spinosissimum* (17.76 ± 0.141 days) (Fig. 2). A Tukey–Kramer comparison of all diet means showed that *A. alliariae* significantly differed from the other two diets but that there was no significant difference between the mixed diet and *C. spinosissimum*. The interaction between family and diet was not significant (Table 2). The plant from which we collected the mothers of the larvae had no influence on development time (Table 2).

Survival was influenced by the diet (Table 3). Survival was highest on the mixed diet (mean of proportion surviving in each family: 0.89 ± 0.025), intermediate on *A. alliariae* (proportion of 0.86 ± 0.025) and lowest on *C. spinosissimum* (proportion of 0.81 ± 0.025) (Fig. 3).

Table 1 ANOVA for growth rates. The family effect was nested within mother origin. Data were ln-transformed before the analyses.

Source of variation	d.f.	SS	*F*	*P*
Mother origin	1	0.0937	1.0611	0.3160
Family (mother origin)	18	1.6169	1.7211	0.0330
Diet	2	3.0391	29.1145	<0.0001
Family × diet (mother origin)	36	2.8145	1.4979	0.0349
Error	446	23.2778		

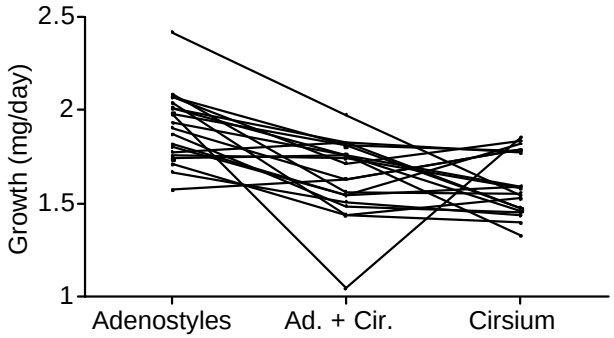

Fig. 1 Growth rates of *O. elongata* larvae from hatching to the end of the third instar. Each line represents the reaction norm of a family and therefore connects the family mean values of each diet.

Table 2 ANOVA for development time. The family effect was nested within mother origin. Data were ln-transformed before the analyses.

Source of variation	d.f.	SS	*F*	*P*
Mother origin	1	0.0001	0.0057	0.9404
Family (mother origin)	18	0.3879	2.1451	0.0042
Diet	2	0.3818	18.9975	<0.0001
Family × diet (mother origin)	36	0.3939	1.0889	0.3368
Error	455	4.5716		

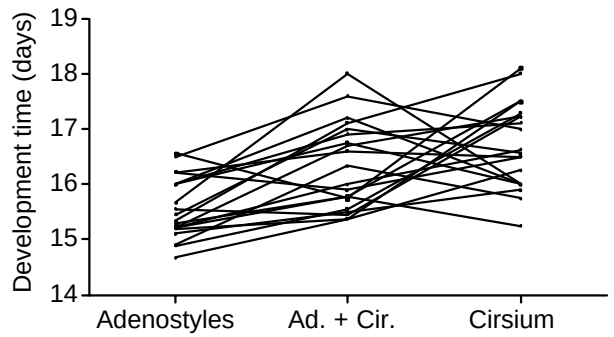

Fig. 2 Development time of *O. elongata* larvae from hatching to the end of the third instar. Each line represents the reaction norm of a family and therefore connects the family mean values of each diet. The crossing of the reaction norms is not significant.

A Tukey–Kramer comparison of all diet means showed that only the difference between the mixed diet and *C. spinosissimum* was significant but not the difference between mixed diet and *A. alliariae* or the one between *A. alliariae* and *C. spinosissimum*. We found genetic variation for survival (family effect) but a nonsignificant interaction between family and diet (Table 3). Figure 3 shows the reaction norms. The plant from which we collected the mothers of the larvae did not influence larval survival (Table 3).

Table 3 ANOVA for survival. The family effect was nested within mother origin. Data were scored as 1 (alive at third moult) or 0 (not surviving until third moult).

Source of variation	d.f.	SS	*F*	*P*
Mother origin	1	0.0017	0.0035	0.9536
Family (mother origin)	18	8.6167	4.2644	<0.0001
Diet	2	0.7300	3.2515	0.0395
Family × diet (mother origin)	36	4.1933	1.0376	0.4116
Error	542	60.8433		

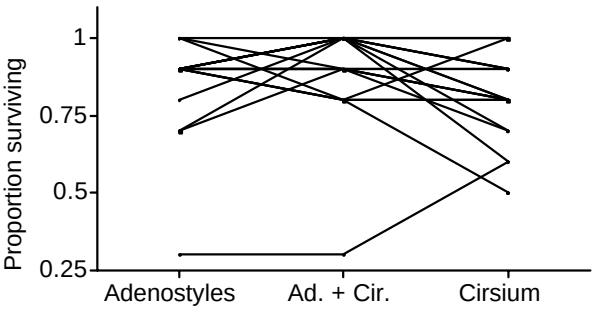

Fig. 3 Survival of *O. elongata* larvae at the end of the third instar. The reaction norms connect the proportions of larvae surviving in each family. The crossing of the reaction norms is not significant.

Table 4 Genetic correlations of each performance trait across diets as estimated by Pearson's correlation coefficients of family means. Bonferroni-corrected *P*-values for differences from zero are given in parentheses. Before analysis, growth rates and development times were ln-transformed and proportions of individuals surviving in each family remained untransformed. *A. a.* = *A. alliariae*, *C. s.* = *C. spinosissimum*.

	Correlations between		
Performance trait	*A. a.* – mixed diet	*A. a.* – *C. s.*	*C. s.* – mixed diet
Growth rate	0.029 (1)	0.065 (1)	−0.210 (1)
Development time	0.463 (0.1194)	0.192 (0.8328)	−0.081 (0.8328)
Survival	0.649 (0.0060)	0.360 (0.1192)	0.480 (0.0646)

Genetic correlations and heritabilities

Table 4 gives the estimates of genetic correlations of each performance character between pairs of diets. Survival was positively correlated between *A. alliariae* and the mixed diet and between *C. spinosissimum* and the mixed diet. All other genetic correlations did not significantly differ from zero. Only two correlations (out of nine) had negative signs (correlations between *C. spinosissimum* and mixed diet for growth rate and development time; Table 4).

The heritabilities of larval survival on *A. alliariae* and on the mixed diet were significantly different from zero, whereby the second estimate was very high (Table 5).

Table 5 Broad sense heritability estimates of larval performance traits on each diet. *P*-values for differences from zero are given in parentheses.

	A. alliariae	Mixed diet	*C. spinosissimum*
Growth rate	0.152 (>0.1)	0.163 (<0.1)	0.167 (<0.1)
Developmental time	0.139 (>0.1)	0.194 (>0.1)	0.018 (>0.1)
Survival	0.081 (<0.05)	1.114 (<0.01)	0.101 (>0.1)

All other within-diet heritability estimates of larval performance gave generally low values which were not significantly different from zero (Table 5).

Discussion

In this study, larvae of *O. elongata* generally performed better on *A. alliariae* (growth rate and development time) and on the mixed diet (survival) than on *C. spinosissimum*. We know that larvae do feed on both plants during their development in our study population, since they hatch on *C. spinosissimum* plants that grow in close proximity to *A. alliariae* and large numbers of larvae move from *C. spinosissimum* to *A. alliariae* during their development (P. Ballabeni *et al*., unpublished data). Furthermore, a preliminary mark–recapture study showed that larvae moved between the two host species within the same day (P. Ballabeni *et al*. unpublished data). Natural selection seems thus to favour larval feeding on both plants and oppose feeding on *C. spinosissimum* alone within this population.

The relatively bad performance on *C. spinosissimum* does not correspond to the natural oviposition preference of this species within the studied population. In separate research we found about 20 times more eggs on *C. spinosissimum* plants than on adjacent *A. alliariae* (P. Ballabeni *et al*., unpublished data). Everything else being equal, phytophagous insects should be selected to lay their eggs on plants that allow best larval performances (Futuyma & Peterson, 1985). However, some additional selective forces acting within natural populations may prevent a positive association between oviposition preference and larval performance. A lack of correspondence between oviposition preference and larval performance in phytophagous insects has been found in several studies (e.g. Roininen & Tahvanainen, 1989; Denno *et al*., 1990; Larsson & Strong, 1992; Fox, 1993). The factor which has received most attention in this context is the role of natural enemies (Price *et al*., 1980; Jefferies & Lawton, 1984; Denno *et al*., 1990; Feder, 1995; Keese, 1997; Rank *et al*., 1998). In the *O. elongata* population of Petit Saint-Bernard we found that eggs had a higher survival on *C. spinosissimum* than on *A. alliariae*, but we do not know whether this was due to differential egg predation between the plants or to some other factors (P. Ballabeni *et al*., unpublished data). The spiny and hairy leaves of *C. spinosissimum* may provide eggs with better adherence, in addition to a hypothetical mechanical protection against predators. If our present performance results do not explain the preference for *C. spinosissimum* for oviposition, they may explain the preference for those *C. spinosissimum* which grow in proximity to *A. alliariae* over those which grow relatively far from *A. alliariae* (P. Ballabeni *et al*., unpublished data). Female *O. elongata* would thus optimize their fitness by ovipositing on the plant which is best for egg survival but close to the plant which allows better larval performance.

The significant family by diet interaction shows genetic variation for reaction norms. Moreover, the lack of significant genetic correlations between the growth rates expressed on the different diets suggests that selection on growth rate can act independently in each environment. These two findings suggest that natural selection has the potential to drive the population towards a hypothetical optimal reaction norm for growth rate, if there was enough genetic variation for this character within each diet (Via & Lande, 1985). However, broad sense heritabilities of the character states expressed in the three different environments were low and not significantly different from zero, although two *P*-values were smaller than 0.1. Future evolution towards an optimal reaction norm of growth rate therefore seems rather unlikely or it should proceed very slowly in the Petit Saint-Bernard population. Alternatively, the nonsignificant heritability estimates, and also their low values which were consistent across diets, suggest that selection has been acting on growth rate in all three environments considered for a relatively long evolutionary time. The reaction norms of growth rate might therefore already be close to optimality in the studied population.

It has been argued that a crossing of reaction norms should be a sufficient condition to favour the evolution of host-specialized genotypes (Fry, 1996). In Fry's model, between-host genetic correlations of performance traits do not need to be negative but only smaller than one for host specialization to evolve, if reaction norms cross (Fry, 1996). However, in spite of the crossing of reaction norms we observed for growth rates, our data suggest that it is unlikely that evolution will drive the *O. elongata* population of the Petit Saint-Bernard towards the formation of specialized host races of the type found, for instance, in the dipteran *Rhagoletis pomonella* (Feder *et al*., 1988; McPheron *et al*., 1988). First, as stated above, growth rate does not seem to posses enough genetic variation within each environment for selection to act. Second, we found a higher larval survival on the mixed diet than on the pure-host diets and a lack of family by diet interaction for survival. This suggests that natural selection should favour the use of a mixed diet.

We did not find any evidence for trade-offs in larval performance across hosts. The only two significant correlations were positive. Only two correlations out of nine had a negative sign and, importantly, all three

correlations between *A. alliariae* and *C. spinosissimum* had positive signs. Even if our estimates of genetic correlations, based on family means, are not very accurate and likely to overestimate true genetic correlations (Roff, 1997; Windig, 1997), it is unlikely that they caused a change of sign from negative to positive. Our results are therefore in agreement with the conventional wisdom that simultaneous adaptation to two hosts should not result in any trade-offs in larval performance between hosts (Via & Lande, 1985; Rausher, 1988). We cannot therefore directly support the prediction by Joshi & Thompson (1995) that trade-offs should appear in a test comparing two normal hosts rather than a normal vs. a novel host. However, Joshi & Thompson's hypothesis predicts that trade-offs should be visible only a certain amount of time after an insect population has incorporated a new plant into its range and genetic equilibrium is approached (Joshi & Thompson, 1995). We do not know how old the associations between *O. elongata* and the two host plants are and whether one host was used earlier than the other within our study population. We can, however, speculate on the base of our heritability estimates. Heritabilities of each performance trait on the two pure hosts had similar values and were lower than the average value given for life history traits in wild animals by Roff (1997, p. 64). Furthermore, our calculations are likely to overestimate real heritabilities since they are broad sense estimates and may therefore be inflated by dominance, epistatic and maternal effects. This reinforces our speculation that the *O. elongata* population of the Petit Saint-Bernard has undergone a relatively long period of selection on both host plants.

The effect of the diet on larval growth we found in this study contrasts with the results found earlier for the same beetle species by Dobler & Rowell-Rahier (1994). They studied larval performance of *O. elongata* from two populations, one of which uses only *A. alliariae* because *C. spinosissimum* is absent, and the other which uses only *C. spinosissimum* because *A. alliariae* is absent. Larvae from both populations grew faster when fed on *C. spinosissimum* then when fed on *A. alliariae*, whereas the effect was stronger for the larvae from the *C. spinosissimum*-only population (Dobler & Rowell-Rahier, 1994). A comparison of our present results and those by Dobler & Rowell-Rahier (1994) shows the existence of a geographical variation in the outcomes of the interaction between *O. elongata* and its hosts that could have consequences on the coevolutionary dynamics of the interacting species (Thompson, 1994, 1997).

To conclude, the *O. elongata* population of the Petit Saint-Bernard perceives its two host plants as a fine-grained environment because larvae feed on both plants during their development (Ballabeni *et al.*, unpublished data). This results in a very reduced genetic variation of the reaction norms of performance characters across hosts and in a very reduced genetic variation of the character states within each host. The genetic patterns observed in this study together with the known oviposition preference (P. Ballabeni *et al.*, unpublished data) suggest that natural selection should maintain feeding on both host plants within the studied population. The evolution of a further specialization towards the use of a single host or the evolution of host specialized genotypes are therefore both unlikely.

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