Genetic architecture for normal and novel host-plant use in two local populations of the herbivorous ladybird beetle, *Epilachna pustulosa*

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Abstract

Trade-offs in host-plant use are thought to promote the evolution of host specificity. However, usually either positive or no genetic correlations have been found. Whereas factors enhancing variation in overall viability have been claimed to mask negative genetic correlations, alternative hypotheses emphasize the sequential changes in genetic correlation in the course of host-range evolution. In this study, the genetic architectures of performances on different hosts were compared in two populations of the herbivorous ladybird beetle, *Epilachna pustulosa*, using three host plants, one being normal for both, one novel for only one population, and the other novel for both populations. The genetic correlations between larval periods on normal hosts were significantly positive whereas those between normal and novel hosts were not different from zero. There was no evidence for reduced genetic variation on the normal host-plants. These results suggest that the host-range is not restricted by the antagonistic genetic associations among exploitation abilities on different plant species, but rather that selection of different host-plants may improve the coordination between genes responsible for the use of different plants.

Introduction

Trade-offs have a central role in theories of life history evolution (Rose, 1982; Stearns, 1992; Falconer & MacKay, 1996). With respect to insect-plant interaction, the genetic correlation between growth performance on one host species and on alternative host, has important implications for the determination of the host-range of the herbivore (Felsenstein, 1981; Rausher, 1984a; Diehl & Bush, 1989). If the performance components on host-plants are negatively correlated, the selection to increase a given performance component on one host-plant will result in a decrease in the analogous component on the other plant species. Genetic trade-offs in host-plant use, therefore, are thought to promote the evolution of host specificity.

With the specific intent of looking for negative genetic correlations, genetic correlations between performance components on one host and an alternative host have been estimated in many herbivorous insects (Jaenike, 1990). However, negative correlations were found in only a limited variety of species (Gould, 1979; Fry, 1990; Via, 1991). Instead of a negative genetic correlation, either positive or no genetic correlations were commonly reported in previous studies (Rausher, 1984b; Via, 1984; Hare & Kennedy, 1986; Futuyma & Philippi, 1987; James *et al.*, 1988; Jaenike, 1989; Karowe, 1990; Fox, 1993; Lu *et al.*, 1997; Ueno *et al.*, 1997, 1999; Balabeni & Rahier, 2000).

Whereas factors that enhance variation in overall viability have been generally regarded as a reason for the lack of negative genetic correlations (Service & Rose, 1985; Charlesworth, 1990; Jaenike, 1990; De Laguerie *et al.*, 1991; Houle, 1991; Price & Schluter, 1991; Fry,

1993), alternative hypotheses have emphasized the sequential changes in the genetic correlations over the course of host-range evolution (Via & Lande, 1985; Rausher, 1988; Thompson, 1994; Joshi & Thompson, 1995). As natural selection changes the frequency of alleles that control host use, the genetic variation and covariation in growth performance on host-plants may change in each generation of selection. As these hypotheses suggest that the history of selection experienced by insect populations on the hosts can influence the current genetic architecture, different stages in the evolution of host use should be distinguished.

For example, the equilibrium trade-off hypothesis proposed by Joshi & Thompson (1995) suggested that for a population using both a novel host and its normal host, trade-offs will become apparent only after the population has evolved to the stage of genetic equilibrium on the two hosts. In general, life history theories indicate that negative genetic correlations among different fitness components will arise as selection either eliminates or fixes the variation in all but the alleles that have antagonistic pleiotropic effects on fitness components (Rose, 1982; Falconer & MacKay, 1996). Thus, subsequent to the initial incorporation of a novel host into the host-range, genetic correlations between performance on novel and normal hosts will become negative over time, as the herbivore population has adapted to both host-plant uses.

Alternatively, trade-offs that once existed may be ameliorated by selection. Epistatic selection may lessen the antagonistic pleiotropic effects by enhancing coordination among loci that affect the performance on different hosts (Via & Lande, 1985; Rausher, 1988; Via, 1991). In this case, an extended period of selection on two hosts may result in a weakening of the negative genetic correlation between performances on both.

When local populations of herbivore species display variation in host-plant use, each population is subject to natural selection for improved ability to use their own host species. The results from comparative studies indicate that the different host use often leads the populations to local adaptation in the use of their own hosts (Via, 1991). Although one genetic architecture may also diverge among populations, reflecting past selection in the use of their hosts, relatively few attempts have been made to compare the within-population genetic architecture of local populations that have adapted so as to use different hosts.

In the present study, we used a quantitative genetic approach to investigate the growth performance of two local populations of the herbivorous ladybird beetle, *Epilachna pustulosa* Kôno (Coleoptera: Coccinellidae). This beetle usually depends on thistle (*Cirsum kamtschaticum*, Asteraceae), but one of the local forms occurs on both thistle and blue cohosh (*Caulophyllum robustum*, Berberidaceae) (Kurosawa, 1953; Katakura, 1974; Hoshikawa, 1984), exhibiting between-populational

variation in host-plant use. Moreover, these beetles can develop normally on potato (*Solanum tuberosum*, Solanaceae), the host-plant of a closely related species, *Epilachna vigintioctomaculata*, although *E. pustulosa* is rarely found in the crop fields. This variation in host-plant use offered us the possibility of distinguishing between the influences of past host-plant use on the genetic architecture of growth performance. Our aims were: (i) to determine whether different host-plant use has led the two local populations to adapt their own hosts, and if so; (ii) to compare the genetic variation and covariation of growth performances on host-plants within and between populations and evaluate the effects of past host use on within-populational genetic architecture; and (iii) to use the above-derived information to determine whether or not the genetic architecture corresponds to the prediction of trade-offs between normal hosts.

Materials and methods

Study organisms

Epilachna pustulosa is one of a group of closely related herbivorous ladybird beetles called the *E. vigintioctomaculata* complex, whose members differ greatly in external morphology and host-plant use in and around Japan (Katakura, 1981, 1997). *E. pustulosa* distributes in Hokkaido, the northernmost island of Japan, where several different morphological forms are recognized (Katakura, 1981). Sapporo-form populations that distribute in southwestern Hokkaido use both thistle and blue cohosh as principal host-plants. There have been only sporadic observations of those beetles on blue cohosh apart from the southwestern Hokkaido populations, and in other districts beetles virtually depend exclusively on thistle despite the distribution of blue cohosh in their regions (Hoshikawa, 1984; Katakura, 1997). Hoshikawa (1984) investigated geographic variations in host preferences of both adult beetles and larvae using 38 populations over Hokkaido. He found a cline in the preference for blue cohosh with the southwestern populations showing the highest preference, although it was consumed only occasionally by northern populations. Between these extremes, Typical-form populations in central Hokkaido showed intermediate preferences for blue cohosh.

In the present study, we used a population of Sapporo-form beetles collected from a suburb of Sapporo in southwestern Hokkaido, and a population of Typical-form beetles from Uryu in central Hokkaido. The two locations are about 80 km apart, and beetles from the two populations have several different morphological characters in their elytral form. These differences in morphology and host-plant use have remained consistent as the Sapporo population was first observed to use both host-plants about 50 years ago (Kurosawa, 1953; Katakura, 1974). Thus, the Sapporo population was

chosen as we expected that both thistle and blue cohosh would be their established normal hosts, whereas virtually no selection is imposed on growth performance on potato. For the Uryu population, thistle is the normal host and selection is imposed on performance only on thistle, yet we expected some extent of feeding response of larvae to blue cohosh leaves to obtain an estimate of their growth performance on a novel host-plant.

Life cycles and experimental procedure

Epilachna pustulosa is a univoltine species. General life cycles of the beetles and their host-plants in Sapporo and vicinity were reported in Katakura (1981). Overwintered adults start to emerge in early May, most stocks of thistle sprout appear in late April, and those of blue cohosh in early May. Initially beetles mainly feed on young thistle leaves and eventually move to the blue cohosh leaves that unfold from mid to late May (Kimura & Katakura, 1986). Beetles copulate on the host-plants repeatedly (Nakano, 1987), and egg clutches are usually oviposited on the underside of the host-plant leaves mainly from early June to early July. The emergence of a new generation of adult beetles starts from late July. The thistle stocks continue growing until July, and stems wither by mid-August, although they develop rosettes by early October. On the other hand, blue cohosh stocks finish growing and bloom in early June. They retain their leaves until late September if not consumed by the beetles. More than half of newly emerging females mate before entering hibernation (Katakura, 1982). There has been no detailed investigation into the life cycles in Uryu, but they appear to have a delayed start and hibernate earlier due to a longer winter period.

Overwintered adults were collected from each population in May and June 1994. Beetles on both thistle and blue cohosh were collected from the Sapporo population, and those on thistle from the Uryu population. The beetles were reared under 22 °C and 16L-8D conditions, which were maintained throughout this experiment. Fresh leaves of thistle were supplied every other day. For each local population, a male-female pair was chosen at random within a local population and confined in a plastic case. Most females would have copulated and stored sperm before overwintering, and additional copulations might have been possible in late spring to early summer before collection. Hence, the experiment was started after at least three copulations were confirmed for each male-female pair. This was to ensure last male paternity; last male sperm precedence (P2) in this species was reported as 0.651–0.827 (Nakano, 1985). Assuming the same sperm precedence pattern for the last three successive copulations, the fertilization success of previous males was expected to be $0.349^3 = 0.0425$ at most. Thus, the experiment was arranged as a split-brood design, though full-sib family brood would have been virtually expected.

The ovipositions of egg clutches by the overwintered generation were checked, and clutches were collected daily. For each female, 45 eggs were collected from several egg clutches. Newly hatched larvae from each egg clutch were divided equally into three. Of these 45 eggs, 15 were reared on thistle, 15 on blue cohosh, and 15 on potato. For each local population, 15 full-sib families were obtained and the experiment was started with 675 larvae from the Sapporo and Uryu population. Larvae were reared individually in a plastic case with a sheet of filter paper to ensure adequate moisture. Fresh host-plant leaves were supplied every other day. Larval development periods were checked daily. On the day of pupation, fresh weight was measured to 0.1 mg with a microbalance.

Statistical analysis

Survival to adulthood was analysed with logistic regression to test for the effects of source population, host-plant and their interaction using PROC CATMOD in SAS (SAS Institute, 1989). To detect the contribution of genotypic effects in local populations, survival was regarded as a threshold trait, with beetles successfully emerged to adults being scored as 1 and those that died before emergence as 0. The threshold values were analysed for each population using PROC GLM with the same procedure as for larval period and pupal weight as in the following except that the effect of sex was not included in the analysis. For larval period and pupal weight, three types of analyses of variance were performed, dividing the data sets in different ways to reveal patterns of genetic viability both within and between the two local populations. In the procedures for analyses of variance, family and host × family interaction were regarded as random effects, whereas sex, population and host were taken to be fixed effects. These analyses were performed for each host-plant pair or each host-plant with ln-transformed data using PROC GLM in SAS. Therefore, the number of tests that involved the same population was controlled by the sequential Bonferroni procedure (Rice, 1989), and all estimates of probability presented in this study are type I error controlled over local populations.

The full model for the present experiment was analysed using population, families nested within a population, host and sex as factors for each pair of host-plants. To detect whether beetles from two local populations, or from families within local population respond differently to host-plants, host × population and host × family (population) interactions were also included in the model. The null hypothesis of the interactions is that the larvae from the two populations or families collected from the local populations respond in the same way to host-plant treatment. If the interactions are significant, then local populations or families from each population have adapted to the particular host-plant.

Data sets were separated for each local population, and mixed model ANOVAs were performed for pairs of host-plants to detect the contribution of factors within each population. The analyses were performed using sex, host, family and host × family as factors. To test the family effect, the host × family interaction was used as the error term. When heritability on each host-plant does not differ, a two-tailed test of family effect divided by interaction indicates whether or not there is significant covariance among host-plants (Fry, 1992).

Each data set was further subdivided into six subsets, each representing a different combination of population and plant species. ANOVAs were performed to evaluate the among-family variance of performance on each host-plant separately. These analyses aimed to detect the potential for responding to the plant environment as well as the effect of past selection on genetic architecture. The PROC VARCOMP in SAS was used to estimate the proportion of phenotypic variation in growth performance attributable to the among- and within-family variances. Between full-sib family variances contain nonadditive sources of genetic variation, including dominance and maternal effects. Although these values should be regarded only as approximations of genetic properties, they nevertheless provide an upper limit of genetic variation. Broad sense heritabilities in each host environment were also calculated using variance components in a one-way ANOVA model. Survival was regarded as a threshold trait, and heritability was calculated following Dempster & Lerner (1950). To compare variance components and heritabilities, the jackknife procedure was used (Roff, 1997; Lynch & Walsh, 1998). To obtain pseudovalues, the genetic parameters were jackknifed with each family omitted once so that the total number of iterations was equal to the number of families. The jackknife estimates of the parameter and its variance were calculated as average and variance of the pseudo-values according to Sokal & Rohlf (1995). To detect whether variance components and heritabilities differ among host-plants within a population, the pseudo-values were then analysed using two-way ANOVA with family and host as factors (Kawecki, 1995). The parameters were compared between the same host-plant across populations by using one-way ANOVA with population as a factor.

The effects of host novelty and quality on variance components were studied following the methods of Kawecki (1995). Survival and pupal weight on host-plant *h* were standardized as [mean ln(x) on host *h*]-[mean ln(x) on all hosts]. For the larval period, as the shorter larval period implies higher performance, the sign of the standardized performance was reversed. The effects of hosts on the variance components were standardized as [σ^2 of trait *x* on host *h*]/[average σ^2 of trait *x* across hosts]. These standardizations of host quality and variance components permitted us to pool the results across traits. To test for the relationship between variance components and host quality, a linear regression of standardized variance components on standardized performance was carried out. Then residuals were analysed with ANOVA to test for the difference between novel and normal hosts. Here, thistle and potato were regarded as normal and novel hosts, respectively, and blue cohosh was treated as a normal host for the Sapporo population and as a novel one for the Uryu population.

The genetic correlation between the growth performances on different host-plants was estimated for each population using the mixed model ANOVA method described by Fry (1992). The variance component for the family effect in the mixed model was used as covariance across host-plants. It was divided by the square root of the cross-product of among-family variance components in a separate one-way ANOVA corresponding to the two host-plants. The variance components estimated by PROC VARCOMP were used. The same procedure as that for variance components was used to compare genetic correlations within and across the population (Knapp *et al.*, 1989; Roff, 1997). In the above calculation of variance components, the TYPE1 option in PROC VARCOMP was used. This option computes the Type I (sequential) sum of squares for each effect, equates each mean square involving only random effects to its expected value, and solves the resulting system of equations (SAS Institute, 1989). Therefore, in this option estimate is not restricted to be positive.

Results

Comparison of growth performances between two local populations

A total of 852 beetles was obtained from 30 families. Growth performances were significantly affected by which host-plant species was fed to the larvae and reflected the host-plant use of the local populations. When the local populations were compared on the two natural host-plants, thistle and blue cohosh, a trend in the present results was characterized by a significant reduction of the Uryu population in growth performances on blue cohosh. Logistic regression indicated a significant host × population interaction for survival (Fig. 1a, Table 1). The Uryu population showed a significantly higher survival rate than the Sapporo population on thistle ($\chi^2_1 = 8.69$, $P < 0.01$), but it was significantly lower than the Sapporo population on blue cohosh ($\chi^2_1 = 45.0$, $P < 0.001$). There was also a difference in survival on potato. Survival of the Sapporo population was significantly higher than that of the Uryu population ($\chi^2_1 = 43.7$, $P < 0.001$).

The two measurements of growth performance, i.e., larval period and pupal weight, also showed a pattern similar to survival. When growth performances on thistle
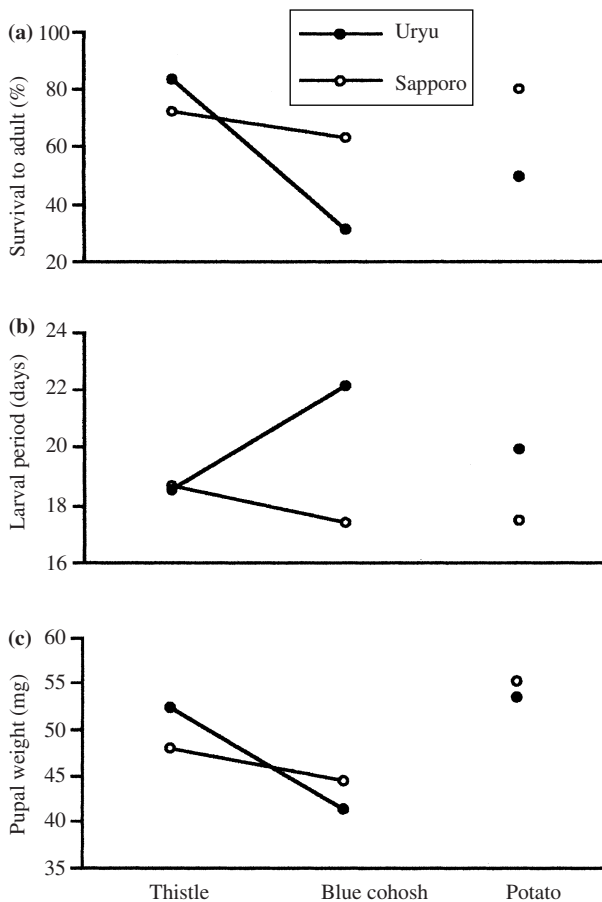
Fig. 1 Host × population interaction plots for survival to adult (a), larval period (b), and pupal weight (c). Two natural host-plants, i.e., thistle and blue cohosh were connected with lines. See Table 1 and 2 for statistical analyses. Standard errors for larval period and pupal weight were smaller than the symbols in all cases.

Table 1 Logistic regression to test for the effects of population, host and host × population on survival rate to adult in the herbivorous ladybird beetle, *Epilachna pustulosa*. Probability was corrected for number of tests by the sequential Bonferroni method.

Source	Thistle–blue cohosh		Thistle–potato		Blue cohosh–potato	
	d.f.	χ^2	d.f.	χ^2	d.f.	χ^2
Intercept	1	55.86*	1	154.44*	1	13.57*
Population	1	4.24	1	4.74	1	84.69*
Host	1	86.11*	1	14.96*	1	31.20*
Host × Population	1	42.24*	1	42.19*	1	0.04

*$P < 0.001$.

larval period and pupal weight (Table 2). Thus, there were significant differences in the two local populations and between families from local populations in their responses to thistle and blue cohosh. Siblings obtained from the Sapporo population, where both thistle and blue cohosh are established normal hosts, developed well on both of the plants. On the other hand, the Uryu population, where thistle is the normal host, showed a significant reduction in growth performance on blue cohosh (Fig. 1b, c). When reared on thistle, the pupal weight of offspring from the Uryu population was greater than that from the Sapporo population ($F_{1,347} = 38.560$, $P < 0.001$), although shorter larval period of the Uryu population was not statistically significant ($F_{1,347} = 1.161$, n.s.). In contrast, when reared on blue cohosh, offspring from the Uryu population showed a lower pupal weight ($F_{1,208} = 19.027$, $P < 0.001$) and a longer larval period ($F_{1,208} = 691.608$, $P < 0.001$) compared with the Sapporo population (Fig. 1b, c). On potato, the novel plant for both populations, siblings from the Sapporo population achieved greater pupal weight ($F_{1,288} = 4.010$, $P < 0.05$) with a shorter larval period ($F_{1,288} = 178.037$, $P < 0.001$) than those from the Uryu population.

Variations in growth performances within local populations

When the Sapporo population was compared for thistle and blue cohosh, the two established normal hosts in this population, those reared on thistle achieved a significantly higher pupal weight than their siblings on blue cohosh (Table 3). At the same time, those reared on thistle showed a significantly longer larval period, and a growth rate [estimated as ln(pupal weight)/day] was significantly higher on blue cohosh (growth rate on thistle = 0.0904; on blue cohosh = 0.0948). Significantly greater pupal weight was observed when beetles were reared on potato compared with their siblings reared on the other two hosts. The larval period on potato was significantly shorter than that on thistle, resulting in a significantly higher growth rate on potato (0.1000) than on the other two hosts. Survival was generally high on the three hosts for the Sapporo population. It was highest on potato and lowest on blue cohosh. There was no significant difference among the host-plants (Table 3).

A significant family main effect was detected in the mixed model ANOVA when analysis was performed for the larval period on thistle and blue cohosh. No significant family effect was detected when analysis was performed for the larval period on potato and thistle, or on potato and blue cohosh, the novel and normal host-plant pairs. For survival and pupal weight, no family main effect was significant. A different response of family to host-plants was indicated as a significant host × family interaction in all host-plant combinations

and blue cohosh were compared, significant effects of host × population and host × family (population) interactions were detected in a full model ANOVA for both

	Larval period			Pupal weight		
Source	$N_{d.f.}$	$D_{d.f.}$	F	$N_{d.f.}$	$D_{d.f.}$	F
Thistle–blue cohosh						
Population	1	31.04	57.702***	1	37.35	0.241
Sex	1	500	0.026	1	500	134.404***
Host	1	39.32	38.326***	1	42.18	118.670***
Family (population)	28	27.98	3.520*	28	27.97	1.473
Host × population	1	39.29	204.059***	1	42.14	31.338***
Host × family (population)	28	500	2.271***	28	500	1.845*
r^2	0.693			0.567		
Thistle–potato						
Population	1	31.62	23.539***	1	34.45	3.099
Sex	1	580	0.769	1	580	176.082***
Host	1	33.72	0.059	1	40.11	48.216***
Family (population)	28	28.00	1.548	28	28.00	1.797
Host × population	1	39.72	44.450***	1	40.10	27.779***
Host × family (population)	28	580	3.222***	28	580	1.590
r^2	0.460			0.435		
Blue cohosh–potato						
Population	1	34.49	316.254***	1	36.21	11.937**
Sex	1	441	0.739	1	441	111.790***
Host	1	33.32	20.043***	1	38.01	276.786***
Family (population)	28	28.00	0.826	28	28.01	1.205
Host × population	1	33.34	23.054***	1	38.04	1.666
Host × family (population)	28	441	3.224***	28	441	1.769
r^2	0.716			0.662		

Table 2 ANOVAs to compare variations in larval period and pupal weight in the herbivorous ladybird beetle, *Epilachna pustulosa* collected from two local populations and reared on thistle, blue cohosh or potato leaves. $N_{d.f.}$ and $D_{d.f.}$ indicate the degree of freedom of mean square used as numerator and denominator to construct F, respectively. Host × family (population) interaction was used as the error term to test for the effects of host, family (population) and host × population. For the effect of population, family (population) was used as error term. Probability was corrected for number of tests by the sequential Bonferroni method. r^2 shows overall fit for the model.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 3 ANOVAs to compare variations in survival, larval period and pupal weight in the herbivorous ladybird beetle, *Epilachna pustulosa* from Sapporo population reared on thistle, blue cohosh or potato leaves. $N_{d.f.}$ and $D_{d.f.}$ indicate the degree of mean square used as numerator and denominator to construct F, respectively. To test for the effects of family and host, host × family interaction was used as the error term. Probability was corrected for number of tests by the sequential Bonferroni method. r^2 shows overall fit for the model.

	Survival			Larval period			Pupal weight		
Source	$N_{d.f.}$	$D_{d.f.}$	F	$N_{d.f.}$	$D_{d.f.}$	F	$N_{d.f.}$	$D_{d.f.}$	F
Thistle–blue cohosh									
Sex				1	272	0.915	1	272	78.688***
Host	1	14	1.82	1	20.30	40.415***	1	20.74	10.736**
Family	14	14	2.87	14	13.98	4.438*	14	13.98	1.082
Host × family	14	420	3.27***	14	272	2.986***	14	272	2.810**
r^2	0.304			0.561			0.448		
Thistle–potato									
Sex				1	310	0.158	1	310	104.095***
Host	1	14	0.74	1	18.94	23.759***	1	20.30	56.426***
Family	14	14	0.81	14	14.00	1.651	14	14.00	1.270
Host × family	14	420	6.61***	14	310	3.248***	14	310	2.590**
r^2	0.277			0.379			0.514		
Blue cohosh–potato									
Sex				1	289	0.497	1	289	77.052***
Host	1	14	6.82	1	15.10	0.097	1	15.20	130.970***
Family	14	14	1.19	14	14.00	1.237	14	14.00	1.009
Host × family	14	420	2.64**	14	289	2.989***	14	289	2.749**
r^2	0.191			0.258			0.651		

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
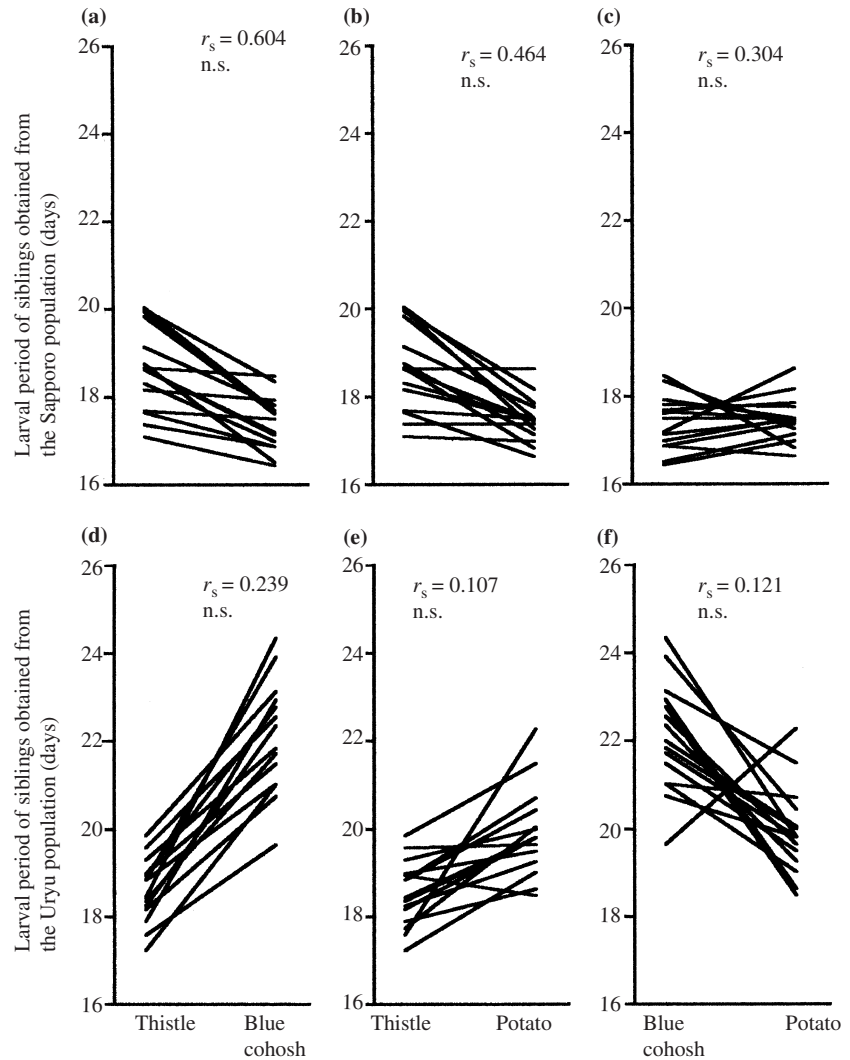
Fig. 2 Host × family interaction plots for larval period of siblings from the Sapporo population (a–c); and the Uryu population (d–f). See Table 3 and 4 for statistical analyses.

(Figs 2 and 3; Table 3). Spearman rank correlation for family means did not detect any significance after the sequential Bonferroni correction (Figs 2 and 3).

For the Uryu population, those reared on thistle exhibited a significantly higher survival together with a shorter larval period and higher pupal weight than their siblings reared on blue cohosh (Table 4). The survival rate was significantly different: the highest survival was on thistle, the lowest on blue cohosh, and intermediate on potato. Pupal weight on blue cohosh was significantly lower than that on thistle or potato, and the larval period was significantly longer than that on either thistle or potato. The growth rate was highest on thistle (growth rate = 0.0931) and lowest on blue cohosh (0.0736), with an intermediate rate on potato (0.0872). The growth rate was significantly different among host-plants.

Family effect was around the 5% significance level when analyses were conducted regarding the larval period on thistle and blue cohosh, for pupal weight on thistle and blue cohosh, and for pupal weight on thistle and potato (Figs 2 and 3; Table 4). However, no family effects were significant when the probability was adjusted for the number of tests performed for the population. Host × family interaction was significant for larval period in all host-plant combinations, but no significant interaction was detected for pupal weight (Figs 2 and 3; Table 4). Spearman rank correlation did not detect any significance (Figs 2 and 3). For survival, the interaction was significant when the analysis was performed with potato and either thistle or blue cohosh (Table 4).

Variance components and genetic correlations

In the Sapporo population, a significant among-family variance was indicated for survival, larval period and
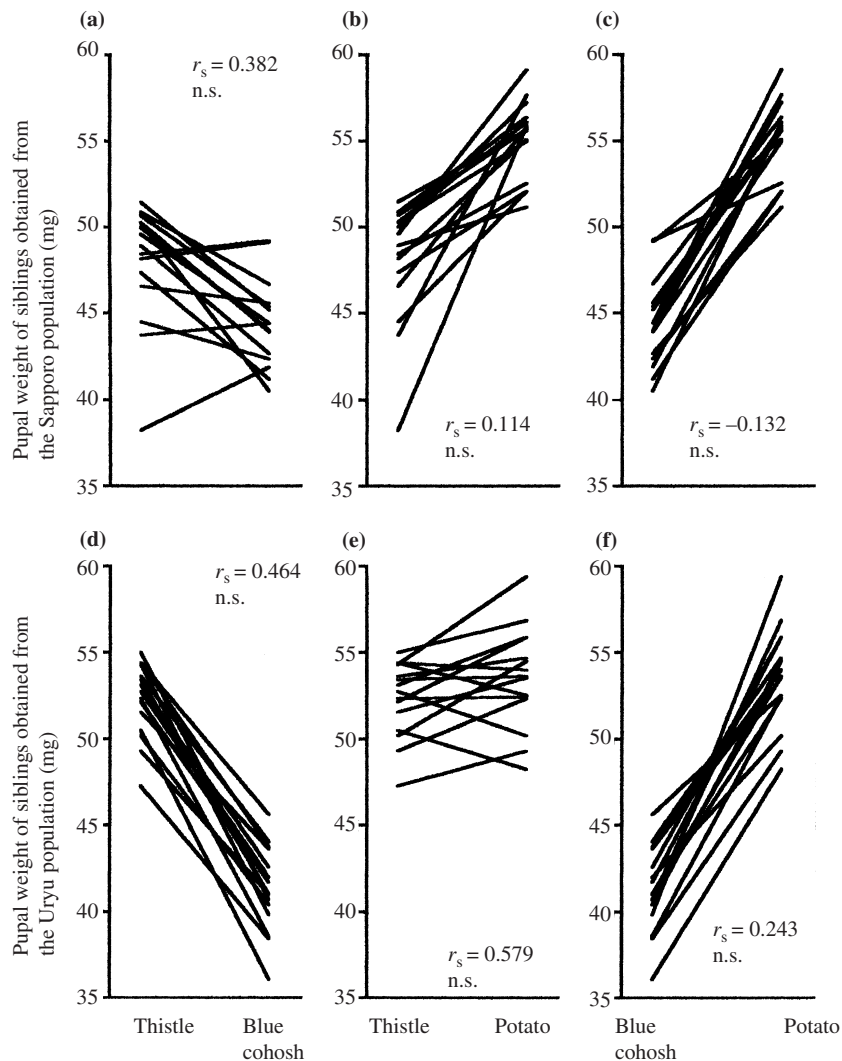
Fig. 3 Host × family interaction plots for pupal weight of siblings from the Sapporo population (a–c); and the Uryu population (d–f). See Table 3 and 4 for statistical analyses.

pupal weight, when tested for each plant separately, except for survival on potato (Fig. 4). Larger among-family variance components were detected for survival and larval period on thistle and blue cohosh than on potato, but the difference was not significant. The within-family variance component was larger for the larval period on potato, and there was a significant difference between potato and blue cohosh (Fig. 4). The variance components resulted in very high and significant broad sense heritabilities of larval period on thistle and blue cohosh, and low heritabilities on potato (Table 5). The estimates, however, were not significantly different among host-plants. For survival, the within-family variance component was significantly smaller on thistle than on blue cohosh. Heritability estimates showed a pattern similar to the larval period, but none was significantly different from zero. For pupal weight, both among- and within-family components were similar

among the three host-plants, and no significant difference was detected (Fig. 4). Heritability estimates for pupal weight on all host-plants were moderate and not significantly different from zero (Table 5).

As for survival in the Uryu population, a significant among-family variance was detected for thistle and potato, but not for blue cohosh. These estimates were not significantly different among host-plants. The within-family variance component was significantly smaller on thistle than on the other two hosts (Fig. 4). Heritability estimates were close to zero for all host-plants (Table 5). For larval period, significant variance was detected for all host-plants, and no difference was suggested for either among- or within-family variance components among host-plants (Fig. 4). Heritability estimates were significantly different from zero only on thistle (Table 5). For pupal weight, significant among-family variance was found only for thistle, and no

Table 4 ANOVAs to compare variations in survival, larval period and pupal weight in the herbivorous ladybird beetle, *Epilachna pustulosa* from Uryu population reared on thistle, blue cohosh or potato leaves. $N_{d.f.}$ and $D_{d.f.}$ indicate the degree of mean square used as numerator and denominator to construct F, respectively. To test for the effects of family and host, host × family interaction was used as the error term. Probability was corrected for number of tests by the sequential Bonferroni method. r^2 shows overall fit for the model

Source	Survival			Larval period			Pupal weight		
	$N_{d.f.}$	$D_{d.f.}$	F	$N_{d.f.}$	$D_{d.f.}$	F	$N_{d.f.}$	$D_{d.f.}$	F
Thistle–blue cohosh									
Sex				1	227	0.944	1	227	55.639***
Host	1	14	118.43***	1	17.36	172.295***	1	21.29	241.004***
Family	14	14	1.39	14	13.98	2.721	14	13.98	2.795
Host × family	14	420	1.56	14	227	1.770*	14	227	0.859
r^2	0.360			0.663			0.643		
Thistle–potato									
Sex				1	269	0.688	1	269	74.292***
Host	1	14	29.47***	1	15.02	20.661***	1	18.15	2.289
Family	14	14	1.05	14	14.01	1.465	14	14.03	3.024
Host × family	14	420	2.45**	14	269	3.151***	14	269	0.813
r^2	0.253			0.388			0.337		
Blue cohosh–potato									
Sex				1	151	0.255	1	151	36.314***
Host	1	14	6.91*	1	15.72	23.959***	1	19.83	160.401***
Family	14	14	0.70	14	14.02	0.581	14	14.06	1.599
Host × family	14	420	2.62**	14	151	2.931**	14	151	0.910
r^2	0.161			0.519			0.671		

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

significant variation was detected for either blue cohosh or potato (Fig. 4). Neither within- or among-family variance components were different among host-plants. No estimate for heritability was significantly different from zero (Table 5).

When the variance components and heritabilities were compared between the same host-plants across the population, a significantly smaller within-family variance component was detected for larval period of the Sapporo population on blue cohosh. For heritability, however, no significant difference was detected. The Sapporo population showed a significantly smaller within-family variance component than the Uryu population when survival and pupal weight on potato were compared.

Linear regression of standardized variance components on standardized performance detected no significant relationship either for among-family variance component ($F_{1,16} = 0.168$, n.s.) or within-family variance component ($F_{1,16} = 0.440$, n.s.). The residuals were analyzed with ANOVA to test for the difference between novel and normal hosts, and the among-family variance component was significantly larger on the normal host than on the novel hosts ($F_{1,16} = 6.223$, $P < 0.05$). The within-family variance component on the normal hosts was smaller than that on the novel hosts, and the result was statistically significant ($F_{1,16} = 6.975$, $P < 0.05$). These statistical results did not change when the standardized variance components rather than the residuals were used in the analysis.

A proper genetic correlation cannot be defined in the absence of genetic variation. Thus, genetic correlations were calculated only between host-plant pairs on both of which significant family effects were detected in separate one-way ANOVAs. The estimates of genetic correlation were positive or close to zero, and no significant negative genetic correlation was found (Table 6). With the significance level indicated by the mixed model ANOVA, a positive genetic correlation for larval period between thistle and blue cohosh, the two normal hosts in the Sapporo population, was significantly different from zero. Although statistically insignificant, a positive genetic correlation was also detected for survival between thistle and blue cohosh in the Sapporo population. No significant difference was detected for either within- or across-populational comparisons.

Discussion

Differential adaptation to thistle and blue cohosh, natural host-plants

Differential responses of the local populations to the two natural host-plants, thistle and blue cohosh, were indicated by significant crossing type of the host × population interaction for all growth performance in the full model ANOVA and logistic regression. The crossing host × population interaction was attributable to a significant reduction in growth performances of the Uryu population on blue cohosh and to comparable growth

(a)

(b)

(c)
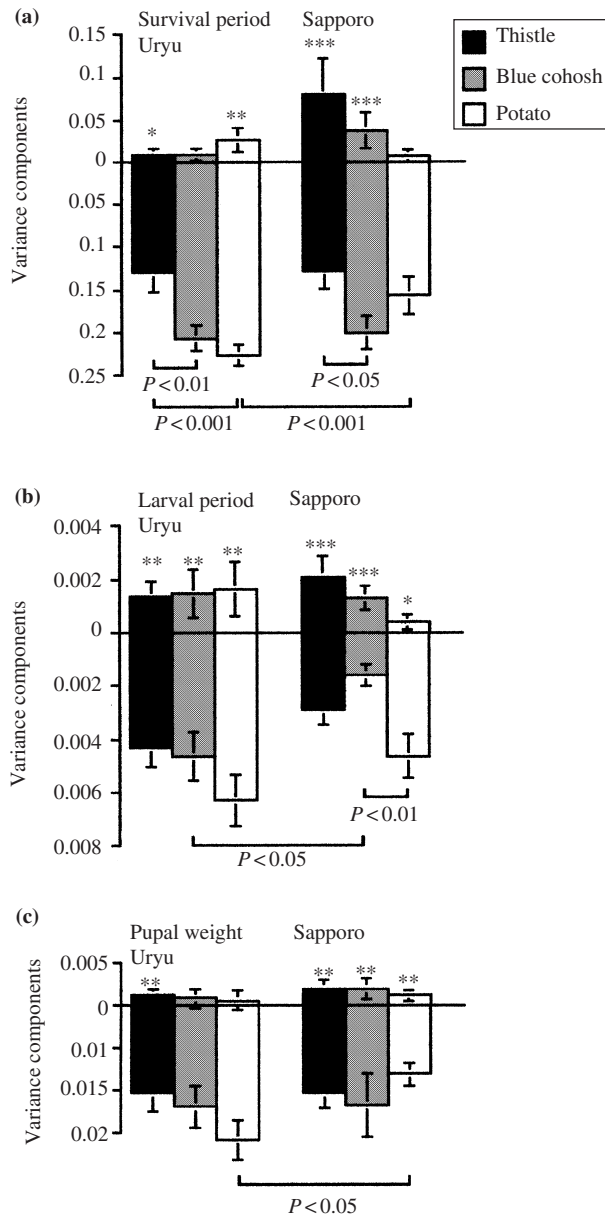
Fig. 4 Jackknife estimates for among-families (above the horizontal bar) and within-family (below the bar) variance components and their standard errors for survival, larval period and pupal weight. Beetles were reared on thistle, blue cohosh or potato leaves. Asterisks show the statistical significance level of the family effect in corresponding one-way ᴀᴠᴏᴠᴀs (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Probability was corrected for number of tests by the sequential Bonferroni method.

Table 5 Jackknife estimate for broad sense heritabilities for survival, larval period and pupal weight of *Epilachna pustulosa*. Varicance components were estimated using TYPE1 option in PROC VAR-COMP (SAS Institute, 1989) with sex as fixed effects. Standard errors of the heritabilities are given in parentheses. Probability was corrected for number of tests by the sequential Bonferroni method.

	Thistle	Blue cohosh	Potato
Sapporo			
Survival	0.421 (0.168)	0.189 (0.101)	0.039 (0.034)
Larval period	0.864 (0.161)***	0.911 (0.259)**	0.174 (0.111)
Pupal weight	0.328 (0.166)	0.284 (0.149)	0.255 (0.125)
Uryu			
Survival	0.049 (0.168)	0.053 (0.041)	0.131 (0.071)
Larval period	0.489 (0.164)*	0.496 (0.282)	0.437 (0.216)
Pupal weight	0.225 (0.166)	0.132 (0.179)	0.080 (0.138)

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 6 Genetic correlation estimate for the herbivorous ladybird beetle, *Epilachna pustulosa* between the same characters across different plant species. Significance test from the mixed model ᴀɴᴏᴠᴀ in Table 3 and 4 were used to mark genetic covariance that is different from zero. Probability was corrected for number of tests by the sequential Bonferroni method. A proper genetic correlation cannot be defined in the absence of genetic variation. Thus, genetic correlation was calculated only between host-plant pairs on both of which significant family effects were detected in separate one-way ᴀɴᴏᴠᴀs.

	Thistle–blue cohosh	Thistle–potato	Blue cohosh–potato
Sapporo			
Survival	0.614	–	–
Larval period	0.758*	0.403	0.173
Pupal weight	–0.056	0.091	–0.067
Uryu			
Survival	–	0.045	–
Larval period	0.600	0.204	–0.356
Pupal weight	–	–	–

*$P < 0.05$.

performances of the Sapporo population on both host-plants. When growth performance of the local populations was compared on thistle and blue cohosh, the Sapporo population always showed better performance than the Uryu population on blue cohosh, whereas the

opposite was true on thistle. These growth performances were measured under identical conditions for the two populations, thus controlling most sources of nongenetic effects. Therefore, the significant and crossing type of host × population interaction suggests a genetic differentiation of local populations because of adaptation to their host-plants. However, because parental beetles from the two populations were fed different host species, it is also possible that host-dependent maternal effects could have contributed to the differential response to host-plants; thus, further investigation must be made to determine whether or not the host-dependent maternal effects generate significant host × population interactions.

Because a shorter development period and higher growth rate on blue cohosh were also observed in the earlier studies conducted on Sapporo populations

(Kurosawa, 1953; Ueno *et al.*, 1997; Yamaga & Ogushi, 1999), the high growth performance on blue cohosh appears to be a consistent characteristic of Sapporo populations. The high affiliation of Sapporo populations to blue cohosh is also suggested by a greater preference for the host-plant in comparison with populations from other localities (Hoshikawa, 1984). These results suggest a positive link at the level of population mean between physiological performance and behavioural preference regarding the use of blue cohosh, and indicate that use of blue cohosh as host-plant has been an important select-ive factor for Sapporo populations.

Within-populational genetic architecture of local populations

Low resolution because of the small sample size in the present study makes it difficult to draw definitive conclusions from the patterns indicated by the estimates of variance components. However, contrary to the above results of local adaptation to their normal host-plants, a generally higher level of heritability was found for performance on their normal host. Heritability estimated for larval period in the Sapporo population was especially high on thistle and blue cohosh. As to the estimation of the larval period on blue cohosh, the high heritability resulted from a reduced within-family variance compo-nent, suggesting that the Sapporo population is better canalized than the Uryu population during development on blue cohosh or when raised on potato.

In the Sapporo population, there was no sign of reduction but rather a larger amount of among-family variation on thistle and blue cohosh than on potato. Moreover, when the different growth performances were standardized and analyzed together, the among-family variance component was significantly greater on the normal hosts. The present results suggest the dominating effect of host-plant novelty on the variance component, but they did not support the prediction from genetic theory that genetic variation will be depleted in the presence of strong selection (Falconer & MacKay, 1996).

The effect of a past history of resource use on the expression of genetic variation in a novel resource environment has been investigated using laboratory populations, with rather mixed results. Transferring to a novel resource environment increased the level of genetic variation in one study (Holloway *et al.*, 1990), however, other studies detected larger genetic variation on normal or closely related hosts than on novel hosts (Kawecki, 1995; Hawthorne, 1997).

For the Sapporo population, different selection regimes on host-plants may serve as an important factor in maintaining genetic variation. Surveys on Sapporo populations revealed that ecological characteristics of thistle and blue cohosh as host environments for *E. pustulosa* are different, with thistles being more abundant and available earlier and longer than blue cohosh (Kimura & Katakura, 1986; Yamaga & Ogushi, 1999). Blue cohosh is often skeltonized by the infestation of the beetles before some of the larvae of *E. pustulosa* complete their development. Because of this depletion, the life history on blue cohosh is characterized by high rate of mortality by a starvation at late larval instars because of intraspecific competition, whereas the major cause of mortality on thistle was reportedly the predation occurring at all larval instars (Kimura & Katakura, 1986; Yamaga & Ogushi, 1999). These selection regimes would result in differential optimal developmental periods on each host-plant, with genotypes of a shorter larval period being especially favoured on blue cohosh. Moreover, the dispersal pattern of ovipositing females is dependent on ecological circumstances such as larval density and plant phenology. Heterogeneity in the resource environ-ment would help in maintaining a genetic variation. Further studies should also be undertaken to determine the effect of variations in the preference of ovipositing females on the maintenance of genetic variation in growth performance.

Trade-offs between growth performance

According to the equilibrium trade-off hypothesis, a negative genetic correlation would be expected between growth performances on host-plants that are established for the herbivore population (Joshi & Thompson, 1995). If a trade-off relation were involved in the host-plant use, it would most likely occur between two characters regarding which selection has been intense, all of whose alleles except for those with a negative association are either fixed or eliminated from the population. Even if this hypothesis is not true in a particular case, a weaker version, i.e., that the rate of loss of variation should be less for antagonistic alleles, might be true.

Although our genetic correlation estimates may not provide a rigorous test for the hypotheses because of the small sample size, the present results indicate a genetic correlation between growth performance on two normal hosts, i.e., thistle and blue cohosh in the Sapporo population, was significantly positive even after applying the sequential Bonferroni test. Furthermore, genetic variation was not eliminated for the traits under selec-tion. This is contrary to what one might expect from the equilibrium trade-off hypothesis. There could be possi-bility for the observation of positive genetic correlation because of the factors that inflate the genetic correlation estimates. However, the estimates of the genetic corre-lations between the two novel hosts or between a novel and normal host were not different from zero, as would be expected in the absence of the simultaneous effect of natural selection on both hosts. This suggests that the inflation of the genetic correlation was unlikely in the present experiment. These results imply that a negative genetic correlation has not played an important role in

the process of adaptation to use different host-plants in the present species, but rather suggest the evolution of a positive genetic correlation.

Natural selection to use host-plants will favour insect genotypes that have improved co-adaptation among genes that are necessary to maximize the growth performance on the host. In the presence of strong selection pressure to use different host-plants simultaneously, improved co-adaptation would be expected not only for the growth performances on each of the host-plants but also for those between the different host-plants. Thus, populations may evolve towards a better coordination across host-plant environments, resulting in the evolution of generalist genotypes. The high growth performance on thistle and blue cohosh and the positive genetic correlation between the two hosts in the Sapporo population might suggest that the past selection of both thistle and blue cohosh has changed the genetic architecture of the local population.

Effects of novel host plants on growth performance

Beetles from both populations showed high growth performance on potato, that is novel to the present ladybird beetles. A phylogenetic study revealed that the use of Solanaceae or Cucurbitaceae is an ancestral character in the group of Epilachna (Kobayashi *et al.*, 1998). It is possible that the physiological ability to use Solanaceae is maintained in the present species, even after beetle populations have adapted so as to specialize on the present host-plants which are totally unrelated to the ancestral host-plant in Solanaceae.

It is also noteworthy that the two local populations showed significant differences in their response to potato. Beetles from the Sapporo population achieved greater pupal weight over a shorter larval period than those from the Uryu population. At the same time, the Sapporo beetles showed smaller within-family variance components than the Uryu population when reared on potato. These results indicate that the Sapporo population had a higher and more stable growth performance on potato, and that there were genetic differences in the potential adaptation to novel host-plants despite the current lack of selection. Such heterogeneous population characteristics may imply that differential adaptation to novel plants occurs at the level of local populations with an interplay between the past history of host-plant use reflected in genetic architecture and present ecological factors such as relative abundance and availability of the host-plants. This heterogeneity may result in the geographic mosaic in evolution of host-plant use (Thompson, 1994).

Acknowledgments

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