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Genetic basis for established and novel host plant use in a herbivorous ladybird beetle, *Epilachna vigintioctomaculata*

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Abstract

Genetic trade-offs in host plant use are thought to promote the evolution of host specificity. Experiments on a range of herbivorous insects, however, have found negative genetic correlation in host plant use in only a limited number of species. To account for the general lack of negative genetic correlation, recent hypotheses advocate that different stages in evolution of host use must be distinguished: initial performance on a novel host in comparison with the established host, and performance on both hosts after the insect population has interacted with both hosts for a long time. The hypotheses suggest that genetic correlation may not necessarily be negative at the initial stage. The present study examines growth performance on both the established and a novel host in a herbivorous ladybird beetle, *Epilachna vigintioctomaculata* Motschulsky (Coccinellidae, Epilachninae). The results show that traits of growth performance across hosts were positively or neutrally correlated, but there was no evidence of a negative genetic correlation. In addition, significant genetic variance of growth performance on each host was detected, suggesting that *E. vigintioctomaculata* can potentially respond to selection for increased performance on both plant species. These results and similar results from experiments on other herbivores suggest that host expansion may not be constrained genetically, at least at the initial stage of host range evolution.

Introduction

Genetic correlations between insect performance across host plants, have important implications for the determination of the host range of herbivorous insects (Rausher, 1984a; Diehl & Bush, 1989). If the performance traits on different host plants are negatively correlated, selection for an increase in a performance trait on one host plant will result in a decrease in the analogous trait on the other plant species. Genetic trade-offs in host plant use are, therefore, thought to promote the evolution of host specificity (Via, 1990).

Experiments estimating the genetic correlation in different host use have been carried out on a range of herbivorous insects (Jaenike, 1990). However, negative correlations have been found in only a limited number of species (Gould, 1979; Fry, 1990; Via, 1991; Mackenzie, 1996). Instead of a negative genetic correlation, positive or no genetic correlations were commonly reported in the 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). To account for this general lack of negative genetic correlations, it has been suggested that antagonistic pleiotropic effects reflecting underlying constraints may be masked by several factors that increase genetic variation in general performance across hosts (Service & Rose, 1985; Houle, 1991; Price & Schluter, 1991; Fry, 1993).

Other recent hypotheses highlight the sequential outcome of genetic correlation in the course of host range evolution (Thompson, 1994; Joshi & Thompson, 1995). These hypotheses suggest that the history of selection experienced by the insect population on the hosts can influence the current genetic structure and therefore that different stages in evolution of host use should be distinguished: initial performance on a novel host in comparison with the established host, and performance on both hosts after the insect population has become adapted to both hosts. Genetic correlation may not necessarily be negative at the initial stage, as alleles affecting the growth performance on the novel host are expected to be randomly associated with alleles affecting the performance on the established host (Joshi & Thompson, 1995). Therefore, in studying genetic correlation between insect performances on different hosts, it is important to consider host plant affiliations in the herbivore population. The experiments reported in this paper represent an initial attempt to examine growth performance on both the established and a novel host in a herbivorous ladybird beetle, *Epilachna vigintioctomaculata*.

E. vigintioctomaculata mainly feeds on *Solanum tuberosum* L. (Solanaceae). Although some additional plants in the Solanaceae family are subsidiarily used by adult beetles, larvae develop almost entirely on *S. tuberosum* (Katakura, 1981). Frequently, beetle populations in Hokkaido (the main northern island of Japan) are found on *Schizopepon bryoniaefolius* Maxim. (Cucurbitaceae) but not in Honshu (the main central island) (Katakura, 1981), exhibiting interpopulational variation in host plant use. The present study compared growth performance on *S. tuberosum* and *S. bryoniaefolius* in a Honshu population. The particular interest in this study was to establish whether genetic factors are responsible for the absence of the beetles on *S. bryoniaefolius* in Honshu populations.

Materials and methods

E. vigintioctomaculata is a univoltine species. Overwintered adults were collected on *S. tuberosum* in a suburb of Niigata City (Honshu) in May 1997. Rearing conditions were 22 °C and L16:D8 throughout this experiment. In excess of 22 males and females were confined as pairs in plastic cases for mating. Virtually all females would have copulated and stored sperm before overwintering (Katakura, 1982) and additional copulations could have also been possible in the late spring before collection. Hence we started the experiment after we confirmed at least three copulations for each female. This was to ensure last male paternity; last male sperm precedence (P2) in closely related species (*E. pustulosa*) was reported as 0.651–0.827 (Nakano, 1985). Assuming the same sperm precedence pattern for the last three successive copulations, the expected fertilization success of previous males are $0.349^3 = 0.0425$ at most. Thus, although the experiment was arranged as a split brood design, full-sib family would have been virtually expected. In the following statistical calculations larvae from each female were regarded as full-sib progenies, assuming complete last male paternity.

For each of the females, 40 eggs were collected from several egg clutches. Of these 40 eggs, 20 were reared on *S. tuberosum* and 20 on *S. bryoniaefolius*. Larvae were reared individually. Fresh host plant leaves were supplied every other day. Larval period was checked daily. On the day of pupation, fresh weight was measured to within 0.1 mg with a microbalance.

A mixed model ANOVA was performed to detect the contribution of factors to the total variation in growth performance, setting family and family × host interaction as random, and sex and host as fixed effects. With this statistical procedure, the variance component due to the family main effect detects the covariance of the performance across the host plants. When divided by the interaction, the family main effects with F-values above 97.5% indicate significant positive genetic covariation, while those with F-values below 2.5% indicate significant negative genetic covariation, at the 5% level (Fry, 1992). Two further ANOVAs were also performed to evaluate the among-family variance of performance on each host plant separately. The analyses were performed to detect the potential response to the plant environment. All ANOVAs were performed using PROC GLM in SAS.

The proportion of phenotypic variation in growth performance attributable to variables was calculated using the restricted maximum likelihood option (REML in PROC VARCOMP, SAS), which estimates the proportion of variables due to each random factor. Because asymptotic variance of each estimate is appropriate for only large samples, significance of the components was determined by the probability obtained from the analogous model ANOVA. Broad sense heritabilities were calculated using the variance components as $h^2 = 2\sigma^2$ (Family)/[σ^2(Family)+σ^2 (Error)]. Standard errors for the heritabilities were estimated after Becker (1984). Between full-sib family variances contain non-additive sources of genetic variation, including dominance and maternal effects. These values should, therefore, be regarded as approximations of genetic properties but they provide an upper limit of genetic variation.

Genetic correlation of the same performance trait across the hosts was estimated using family means

Table 1. Percentage survival and means (±s.e.) for larval period and pupal weight in a herbivorous ladybird beetle, *Epilachna vigintioctomaculata* reared on *Solanum tuberosum* or *Schizopepon bryoniaefolius*. Sample sizes are given in parentheses

	Host	
	Solanum tuberosum	*Schizopepon bryoniaefolius*
Larval period (days)		
Male	14.9 ± 0.1 (168)	17.5 ± 0.2 (141)
Female	14.9 ± 0.1 (148)	17.8 ± 0.1 (137)
Pupal weight (mg)		
Male	40.2 ± 0.4 (168)	33.8 ± 0.3 (141)
Female	43.9 ± 0.4 (148)	36.9 ± 0.3 (137)
Survival to adult (%)	71.8	63.2

Table 2. Results of log-likelihood-ratio analysis for survivorship in a herbivorous ladybird beetle, *Epilachna vigintioctomaculata* reared on *Solanum tuberosum* or *Schizopepon bryoniaefolius*

Source	d.f.	G	P
Host	1	7.50	0.006
Family	21	62.4	<0.001
Family × Host	21	22.4	0.375

as $r_m = \mathrm{Cov}(X, Y)/[\mathrm{Var}(X)\mathrm{Var}(Y)]^{1/2}$, where $\mathrm{Cov}(X, Y)$ is the covariance of family mean of the trait reared on one host plant and family mean of the same trait reared on the other host, and $\mathrm{Var}(X)$ is the variance of the family mean on one host plant (Via, 1984). Genetic correlation was also estimated using variance components obtained from REML in PROC VARCOMP adopted to the mixed model. Here genetic correlation was estimated as $r_G = \sigma^2(\text{Family})/[\sigma^2(\text{Family} \times \text{Host})+\sigma^2\ (\text{Family})]$ (Fry, 1992). Jackknife procedure was used to calculate the correlation with their standard errors (Sokal & Rohlf, 1995). Here the correlation was jackknifed with each family omitted once so that the total number of iterations was equal to the number of families. The correlation coefficients were z-transformed for jackknifing procedures and transformed back to the coefficient scale after obtaining 95% confidence intervals for z-values.

Results

Totally, 594 beetles were obtained from 22 families. Log-likelihood-ratio analysis detected a significant effect of host plant on survivorship. Individuals reared on *S. bryoniaefolius* showed a higher mortality rate than those reared on *S. tuberosum* (Tables 1, 2). Different survivorship rates among families were detected, but there was no evidence for family × host interaction (Table 2). Larval period and pupal weight were also significantly influenced by host plants (Table 1). Those reared on *S. tuberosum* showed a shorter larval period than those reared on *S. bryoniaefolius*. Larvae which were reared on *S. tuberosum* achieved a heavier pupal weight than their siblings which were raised on *S. bryoniaefolius*. This between plant difference in overall tendency was observed in both males and females. Females were significantly heavier than male siblings, while larval periods were not significantly different between the sexes (Tables 1, 3).

The pattern of contribution of factors to the variation in performance was different for the larval period and for pupal weight. The mixed model ANOVAs detected significant family × host interaction for the larval period but not for pupal weight. By contrast, the family main effect in the mixed model ANOVAs, which indicates genetic covariance across hosts, was not significant for the larval period but was significantly positive for pupal weight with a two-tailed test (Table 3).

The genetic correlation calculated with variance components reflected the above results of the mixed model ANOVAs (Table 4). The genetic correlations for both components of growth performances were

Table 3. ANOVAs to compare variations in larval period and pupal weight of *Epilachna vigintioctomaculata* reared on *Solanum tuberosum* or *Schizopepon bryoniaefolius*. r_2 shows the fit of the overall model

	Larval period				Pupal weight			
Source	d.f.	MS	F	P	d.f.	MS	F	P
Sex	1	0.007	1.202	0.2735	1	0.962	46.249	$<$0.0001
Host	1	3.599	287.227	$<$0.0001	1	3.901	133.974	$<$0.0001
Family	21	0.021	1.629	0.1359	21	0.076	2.570	0.0179
Family × Host	21	0.013	2.160	0.0021	21	0.029	1.416	0.1036
Error	549	0.006			549	0.021		
r^2	0.587				0.392			

Table 4. Genetic correlation estimates of *Epilachna vigintioctomaculata* between the same characters across different plant species with their 95% confident limits in parentheses. Genetic correlations were estimated using variance and covariance of the family means (r_m) and using variance components of the mixed model (r_G)

	Larval period	Pupal weight
r_m	0.320 (−0.111, 0.649)	0.560 (0.015, 0.849)
r_G	0.378 (−0.045, 0.686)	0.641 (0.181, 0.871)

positive. In particular, the genetic correlation was highly positive for pupal weight. Estimated values of family mean genetic correlations (r_m) were in agreement with those based on variance components (r_G).

From the ANOVA performed for growth performance on *S. tuberosum* and *S. bryoniaefolius* separately, significant among-family variation was detected in each case (Table 5). ANOVA detected significant among-family variance for both the larval period and pupal weight. Variance components attributable to family were larger for beetles reared on *S. bryoniaefolius* than for those reared on *S. tuberosum* (Table 6). Broad sense heritabilities were moderate for performance traits on *S. bryoniaefolius*, while rather lower heritabilities were obtained for both the larval period and pupal weight on *S. tuberosum* (Table 6).

Discussion

The purpose of this study was to see how traits of growth performance on the established host are genetically correlated with analogous traits on a novel host. The results showed that performance traits were significantly positively or neutrally correlated, suggesting that selection for increased performance on one host will not constrain, but will rather facilitate evolution of increased performance on the other host. Despite the difference in host plants, the present study found no evidence for genetic trade-offs in host plant use.

In addition, significant among-family variance on each host was detected when statistical analyses were made for each host plant, suggesting that *E. vigintioctomaculata* can potentially respond to selection for increased performance on both plant species. Rather small among-family variance components were found on *S. tuberosum* when compared to those on *S. bryoniaefolius*. This result suggests that, to a substantial extent, selection on *S. tuberosum* has been imposed and that the response to further selection on the established host will evolve less readily due to reduced genetic variation. In contrast, larger among-family variance components on *S. bryoniaefolius*, suggest that the present population would easily respond to selection for increased performance on the novel host.

The above results indicate that the ability to exploit the novel host is not likely to be genetically constrained either in terms of genetic correlation or of genetic variation. When experiments were made on established and novel host plants, the results from previous experiments on other herbivorous insects indicate positive or no genetic correlation (Rausher, 1984b; Via, 1984; Hare & Kennedy, 1986; James et al., 1988; Karowe, 1990; Fox, 1993), and significant genetic variation in growth performance on each plant (Rausher, 1984b; Hare & Kennedy, 1986). These and the present results suggest that host expansion may not be constrained genetically at least at the initial stage of host range evolution.

It is important to note, however, that the experimental conditions may have been responsible for the absence of genetic constraints. In the studies com-

Table 5. ANOVAs to compare variations in larval period and pupal weight of *Epilachna vigintioctomaculata* reared on *Solanum tuberosum* or *Schizopepon bryoniaefolius*. r_2 shows the fit of the overall model

Solanum tuberosum	Larval period				Pupal weight			
Source	d.f.	MS (×100)	F	P	d.f.	MS	F	P
Sex	1	0.002	0.005	0.9455	1	0.436	14.069	0.0002
Family	21	0.685	1.621	0.0438	21	0.057	1.841	0.0149
Error	293	0.422			293	0.031		
r^2	0.104				0.156			

Schizopepon bryoniaefolius	Larval period				Pupal weight			
Source	d.f.	MS	F	P	d.f.	MS	F	P
Sex	1	0.017	2.133	0.1454	1	0.535	58.325	$<$0.0001
Family	21	0.025	3.187	$<$0.0001	21	0.051	5.507	$<$0.0001
Error	255	0.008			255	0.009		
r^2	0.217				0.409			

Table 6. Variance components (×100) for analyses in Table 5, and broad sense heritabilities calculated using the variance components. Variance components were estimated using REML with host and sex as fixed effects. Significance tests from the ANOVA in Table 5 were used to mark variance components that are greater than zero. Percentages of the variance components and standard errors of the heritabilities are given in parentheses

	Larval period		Pupal weight	
	Solanum tuberosum	*Schizopepon bryoniaefolius*	*Solanum tuberosum*	*Schizopepon bryoniaefolius*
Family	0.018 (4.0)*	0.142 (15.2)**	0.180 (5.5)*	0.353 (27.7)**
Error	0.424 (96.0)	0.788 (84.8)	3.102 (94.5)	0.918 (72.3)
Heritability	0.081 (0.066)	0.305 (0.120)	0.110 (0.073)	0.555 (0.155)

*P $<$ 0.05; **P $<$ 0.0001.

paring growth performance on established and novel hosts, novel hosts on which insects have been usually tested are the hosts of the conspecific or congeneric population. It is possible that an absence of genetic constraint is what permits the use of these hosts in the conspecific or congeneric population. Therefore, that the hosts of related population can potentially be incorporated into host range, may not necessarily indicate the absence of genetic constraints that prevent the incorporation of other plants.

One aim of this study was to establish whether genetic factors are responsible for the absence of the beetles on *S. bryoniaefolius* in Honshu populations. In this study, there was no evidence for genetic constraints for the Niigata population in expanding its host range to include *S. bryoniaefolius*. The results suggest that behavioural and ecological factors may be the primary influence on host plant use. Investigations of these factors in natural populations will be necessary in future studies.

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