Genetic variation in larval period and pupal mass in an aphidophagous ladybird beetle (*Harmonia axyridis*) reared in different environments

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Abstract

Genetic trade-offs for host plant use are hypothesized to facilitate the diversification of insect populations through specialization to their host plants. Previous studies mainly estimated the architecture of genetic variances and covariances in herbivorous species with discrete and limited types of host species. In contrast to herbivores, the relative abundance of resources for predatory species fluctuates in time and space, causing a more unpredictable encounter with prey species. The ecological characteristics of resource use might result in a differential mode of selection for herbivorous and predatory species, which could be reflected in a differential genetic architecture of developmental traits such as the duration of larval stage (henceforth referred to as larval period) and size of pupa (measured as pupal weight). This paper presents results from a study on the genetic architecture of larval period and pupal mass of an aphidophagous ladybird beetle, *Harmonia axyridis* Pallas, in different resource environments. Beetles reared on *Acyrthosiphon pisum* (Harris) showed a shorter developmental period and a heavier pupal mass than their siblings on *Aphis craccivora* Koch or on artificial diet, while the average larval period and pupal mass on *A. craccivora* and the artificial diet were similar. Further analyses of the genetic architecture suggest that the developmental traits on the two aphid species are genetically correlated, while there are only weak or no genetic correlations between these two traits on the two aphid preys and the artificial diet. Thus, the results suggest that the patterns of genotypic relationships between developmental traits differ from the phenotypic ones. The effects of past selection on the genetic architecture and the possible cause of the genetic correlation are discussed, as well as consequences for mass rearing for biological control.

Introduction

Genetic correlation between traits expressed in different environments has been a central focus in evolutionary genetics (Roff, 1992; Stearns, 1992). As the genetic correlation between a trait expressed in one environment and the analogous trait in another environment affects the rate and direction of trait change (Via & Lande, 1985), studies on the architecture of genetic variances and covariances are important in understanding evolutionary consequences. If a fitness component in one environment is negatively correlated with the corresponding component in the another, selection for a trait increasing the fitness in one environment will result in a decrease in the other. This fitness trade-off may furthermore lead to the evolution of environmentally-specialized genotypes, which are fitter in one particular environment than other genotypes. On the other hand, with a positive genetic correlation, selection could lead to a population where genotypes with a high fitness on one host would also show a high fitness on the other. Thus, a difference in environment would then cause little difference in fitness among genotypes.

Specifically, because genetic trade-offs for host plant use were thought to facilitate the diversification of insect populations through specialization to their host plants, extensive studies have been carried out to estimate genetic architecture in herbivorous species (Futuyma & Moreno,

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1988; Jaenike, 1990; Via, 1990). The results showed mainly positive or no genetic correlations (Rausher, 1984; 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, 2001; Ballabeni & Rahier, 2000). The exceptions have been found in highly specialized species (Gould, 1979; Fry, 1990; Via, 1991). As a consequence of the focus of research on negative genetic correlations, herbivorous species with discrete host use were generally chosen in previous studies. Except for a few cases (Ballabeni & Rahier, 2000), host plants are dispersed, which virtually prevents the herbivore larvae from simultaneous use of different host plants. The present understanding of the genetic architecture of developmental traits, consequently, tends to be restricted to herbivorous species in a coarse-grained environment with a specialized resource range.

In contrast to herbivores, the larvae of predatory species, especially those that do not receive any type of parental care, must move more to exploit prey. For those species, the relative abundance of resources fluctuates in time and space, thus causing their encounters with prey species to be more unpredictable. Therefore, the resource environment of predatory species is generally more fine-grained with a broader range of resources, although the actual mode of resource use falls within a continuum between the two extremes. The ecological characteristics of resource use might result in a differential mode of selection for herbivorous and predatory species, which could be reflected in a different genetic architecture of developmental traits. Thus far, however, few researches have been conducted on the genetic architecture of developmental traits in predatory species.

The present study reports results on the genetic architecture of larval period and pupal mass in different resource environments, in an aphidophagous ladybird beetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). This species feeds on a wide range of aphid species (Osawa, 1991). Most aphids are specific to host plant species and to host plant parts available in a restricted period of host plant phenology (Kawauchi, 1990; Osawa, 1991). As *H. axyridis* shows no tendency to choose aphid species when they oviposit (Okamoto, 1978), the resource environment of the ladybird beetle varies considerably though both time and space, depending primarily on the various ecological factors at the site of oviposition. Although it is difficult to know the degree to which the resources are heterogeneous under natural conditions during the development of individual larvae, in a poor resource environment, exploiting different resources would be very likely since the beetles search for prey at random, and selection of the plant they climb in is by chance (Kawai, 1976). Altogether, the ecological characteristics of the ladybird beetle are expected to be suitable for the study of the genetic architecture of developmental traits in a predatory species.

Materials and methods

Harmonia axyridis overwinters in the adult stage, and begins oviposition in the next spring. Pupae of a new generation were collected from a suburb of Gifu City, around the centre of the main island of Japan, in June 1994. They were kept individually under L16:D8 conditions at 25 °C. These rearing conditions were maintained throughout the experiment. Emerged adults were fed with an artificial diet made from freeze-dried honeybee larvae (Matsuka & Okada, 1975; Matsuka & Niijima, 1985). The adult sex was identified, and males and females were paired. After copulation, each female was kept individually and egg clutches were checked daily. For each female, 45 full-sib individuals were collected from several egg clutches. Newly hatched larvae were divided into three equal parts. Of 45 larvae, 15 were reared on *Acyrthosiphon pisum* (Harris), 15 on *Aphis craccivora* Koch, and the other 15 were reared on artificial diet. Based on prior work (Okamoto, 1978), *Acyrthosiphon pisum* and *A. craccivora* were chosen as favorable and poor resource species, respectively. An aphid colony on a bean plant was offered to each beetle larva. A bean plant seedling of about 10 cm length was fixed in a glass tube by binding cotton paper around the stem. To prevent plant growth, its root was cut off, and wet cotton was placed at the bottom of the glass tube to retain adequate moisture. Under these conditions, aphids introduced on the bean plant can develop into a colony. When the aphids were eaten or the plant started withering, the beetle larva was moved to a new aphid colony. Larvae were checked for pupation every day, and after pupation, each individual was weighed to an accuracy of 0.01 mg with a microbalance.

A mixed model ANOVA was used to detect the contribution of the factors to the total variation of larval period and pupal mass with family and family × resource interaction as random effects, and sex and resource as fixed effects. This analysis was performed with the log-transformed data of larval period and pupal mass, and for each possible pair of the three resources. When heritability in each resource environment does not differ, the variance component due to the family main effect detects the covariance of the developmental trait across the resource environments in the present procedure. 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). Three further

ANOVAs were also performed to evaluate the among-family variance of larval period and pupal mass in each resource environment separately. All ANOVAs were performed using the PROC GLM routine in SAS software.

The PROC VARCOMP in SAS was used to estimate the proportion of phenotypic variation in larval period and pupal mass attributable to the among- and within-family variances. Broad sense heritabilities were calculated using the variance components as $h^2 = 2\sigma^2(\text{among-family})/[\sigma^2(\text{among-family}) + \sigma^2(\text{within-family})]$ (Becker, 1984; Lynch & Walsh, 1998). Among full-sib family variances contain nonadditive 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 to the genetic variation. To calculate the variation of the estimated parameters in each resource environment, a jackknife procedure was used (Lynch & Walsh, 1998). Here, estimates were jack-knifed with one family omitted each time so that the total number of iterations was equal to the number of families (Sokal & Rohlf, 1995). To detect whether variance components and heritabilities differed across resource environments, pseudo-values were analyzed with a two-way ANOVA with family and resource environment as factors.

Genetic correlation of the same developmental trait across the resources was estimated using the family means as $r_m = \text{Cov}(X,Y)/[\text{Var}(X)\text{Var}(Y)]^{1/2}$, where Cov(X,Y) is the covariance of the family mean of the trait reared in one resource environment and the family mean of the same trait reared in the other resource environment, and Var(X) is the variance of the family mean in one environment (Via, 1984; Via & Conner, 1995). The jackknife procedure was used to calculate the correlations with their standard errors. In the above calculation of variance components, the TYPE1 option in PROC VARCOMP was used because in this option the estimate is not restricted to be positive.

Table 1 Means ± SE for larval period and pupal mass of *Harmonia axyridis* reared on *Acyrthosiphon pisum*, *Aphis craccivora*, or artificial diet. Sample sizes are given in parentheses

	Resource		
	A. pisum	*A. craccivora*	Artificial diet
Larval period (days)			
Male	13.8 ± 0.5 (44)	18.8 ± 0.7 (32)	19.7 ± 0.6 (32)
Female	13.1 ± 0.3 (45)	20.6 ± 0.8 (22)	21.1 ± 0.6 (41)
Pupal mass (mg)			
Male	27.2 ± 0.5 (44)	19.6 ± 0.6 (32)	20.9 ± 0.9 (32)
Female	28.5 ± 0.7 (45)	22.0 ± 0.9 (22)	23.1 ± 0.7 (41)

Results

Larval period and pupal mass were significantly influenced by resources in both males and females (Tables 1 and 2).

Table 2 ANOVAS to compare variation in larval period and pupal mass of *Harmonia axyridis* reared on *Acyrthosiphon pisum*, *Aphis craccivora*, or artificial diet. r^2 shows overall fit of the model

	Larval period				Pupal mass			
Source	$N_{d.f.}$	$D_{d.f.}$	F	P	$N_{d.f.}$	$D_{d.f.}$	F	P
A. pisum–A. craccivora								
Sex	1	118	1.189	0.2778	1	118	6.476	0.0122
Resource	1	11.41	72.706	< 0.0001	1	12.70	99.794	< 0.0001
Family	11	11.00	3.423	0.0526	11	10.98	1.376	0.3031
Resource × Family	11	118	4.388	< 0.0001	11	118	1.096	0.3707
r^2	0.825				0.586			
A. pisum–artificial diet								
Sex	1	137	0.231	0.6315	1	137	7.605	0.0066
Resource	1	11.04	29.545	0.0002	1	11.35	35.768	< 0.0001
Family	11	11.00	0.460	0.8930	11	10.99	1.695	0.1977
Resource × Family	11	137	18.853	< 0.0001	11	137	2.223	0.0165
r^2	0.870				0.524			
A. craccivora–artificial diet								
Sex	1	102	0.733	0.3940	1	102	13.287	0.0004
Resource	1	11.26	0.288	0.6018	1	11.61	0.557	0.4704
Family	11	10.98	0.654	0.7535	11	10.96	0.723	0.7003
Resource × Family	11	102	5.583	< 0.0001	11	102	2.397	0.0109
r^2	0.530				0.404			

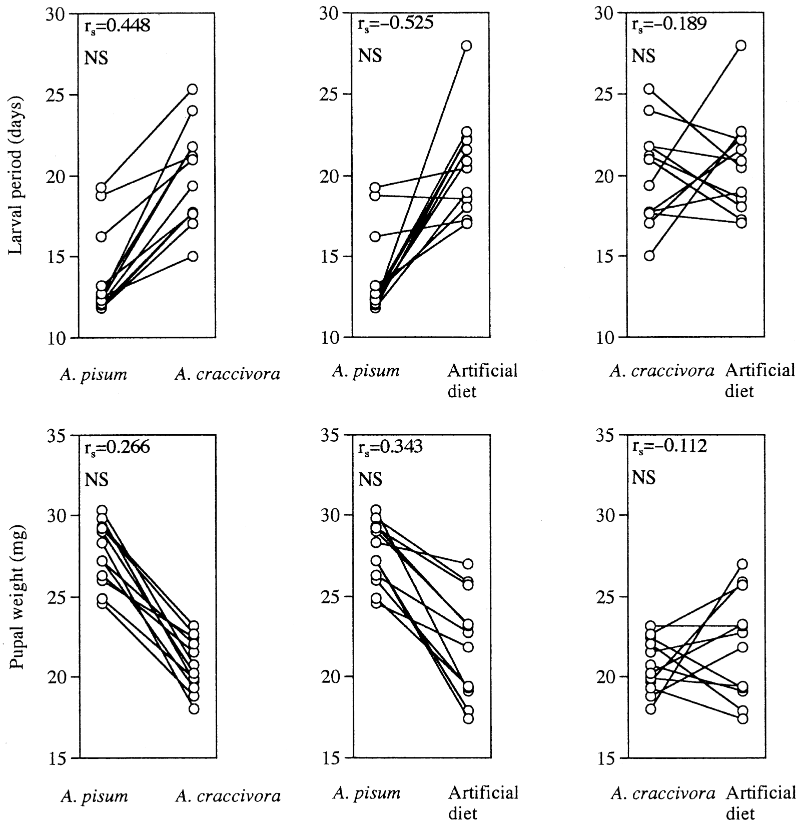

Figure 1 Variation among full-sib families of *Harmonia axyridis* for larval period and pupal mass. Least-squares mean adjusted for effect of sex is shown for each family reared on *Acyrthosiphon pisum*, *Aphis craccivora*, or artificial diet. Lines connect the means for each family. Non-parallelism is the expression of family × resource interaction. See also Table 2 for the statistical results for the interaction. Spearman rank correlation(r_s) was not significant (NS) for all cases.

The beetles reared on *A. pisum* showed a shorter developmental period and a heavier pupal mass than their siblings on *A. craccivora* or on the artificial diet. Between individuals raised on *A. craccivora* and on the artificial diet, no significant difference was detected. Females were significantly larger and heavier than males, while larval period was not significantly different between sexes. Thus, females attained a larger body size than males although they had about the same developmental period as males.

The mixed ANOVA detected a significant family × resource interaction for larval period in all resource environment pairs. When the analysis was performed for larval period on the two aphid species, family main effect divided by the interaction term was positive but insignificant with a two-tailed statistical test ($P = 0.0526$). For the other two pairs of resource combinations, where the artificial diet was involved, F-statistics for the family effect divided by the interaction were less than one and not statistically significant (Table 2). For pupal mass, a significant family × resource interaction was detected for the two pairs of resource combinations, the artificial diet and either of the aphid species. For pupal mass on *A. pisum* and *A. craccivora*, no significant family × resource interaction was detected. No family main effect over interaction was significant.

The interaction between family and resource environment was also indicated by the rank correlation (Figure 1). Although Spearman rank correlations were not statistically significant, substantial changes in the relative rank position were observed in some families, especially when the analysis was performed for the developmental traits on the artificial diet and either of the two aphid species, suggesting a different response of the families to the aphid species and the artificial diet.

A separate ANOVA for individuals reared on the artificial diet indicated significant among-family variation for both larval period and pupal mass, while the analogous analyses for individuals reared on *A. pisum* and *A. craccivora* only detected significant variation for larval periods (Table 3). The further analysis on variance components suggested different patterns among resource environments (Table 4). For the larval period, the among-family variance component was not different across the resource environments (d.f. = 2,22, $F = 0.247$, $P = 0.7831$), whereas a significant difference was detected for the within-family variance component (d.f. = 2,22, $F = 8.098$, $P = 0.0023$). The within-family variance component detected for the beetles reared on *A. pisum* was significantly smaller than the analogous component for their siblings on *A. craccivora*, even after the probability was corrected by the sequential

Table 3 ANOVAS to compare variation in larval period and pupal mass of *Harmonia axyridis* reared on *Acyrthosiphon pisum*, *Aphis craccivora* or artificial diet. r^2 shows overall fit of the model

	Larval period				Pupal mass			
Source	$N_{d.f.}$	$D_{d.f.}$	F	P	$N_{d.f.}$	$D_{d.f.}$	F	P
A. pisum								
Sex	1	76	0.252	0.6170	1	76	1.296	0.2585
Family	11	76	26.115	< 0.0001	11	76	1.443	0.1715
r^2	0.794				0.189			
A. craccivora								
Sex	1	41	0.897	0.3491	1	41	6.467	0.0149
Family	11	41	3.082	0.0043	11	41	1.180	0.3307
r^2	0.484				0.316			
Artificial diet								
Sex	1	60	0.055	0.8155	1	60	6.981	0.0105
Family	11	60	6.590	< 0.0001	11	60	3.524	0.0008
r^2	0.561				0.424			

Bonferroni procedure for the number of tests (P = 0.0017). For pupal mass, the among-family variance component on the artificial diet was larger than that on the two aphid resources, but the difference was not statistically significant (d.f. = 2,22, F = 3.425, P = 0.0507). There was no significant difference in the within-family variance component across the environments (d.f. = 2,22, F = 1.602, P = 0.2240).

The heritability estimates for larval period were quite high in all resource environments (Table 4). For pupal mass, only siblings reared on the artificial diet showed a high heritability, and heritability estimates were low when siblings on the two aphid species were analyzed. The estimates, however, were not significantly different for one another (larval period: d.f. = 2,22, F = 2.316, P = 0.1222; pupal mass: d.f. = 2,22, F = 1.674, P = 0.2106).

The genetic correlation between the larval period on *A. pisum* and that on *A. craccivora* was positive, whereas negative correlations were estimated between the aphid species and the artificial diet (Table 5). A two-way ANOVA performed on the pseudo-values detected a significant difference among the three correlations (d.f. = 2,22, F = 4.100, P = 0.0307) and showed that the correlation between *A. pisum* and *A. craccivora* was significantly larger than that between *A. pisum* and the artificial diet (P = 0.0366). For pupal mass, estimates for genetic correlation were close to zero and there was no difference amongst them (d.f. = 2,22, F = 1.722, P = 0.1339).

Discussion

Significant effects of food resources on larval period and pupal mass were indicated by the present results for the ladybird beetle at the phenotypic level. Beetles reared on *A. pisum* showed a shorter developmental period and heavier pupal mass than their siblings on *A. craccivora* or on the artificial diet, while the average larval period and pupal mass on *A. craccivora* and the artificial diet were similar to each other. A further analysis of the genetic architecture of the developmental traits, however, suggested that the patterns in the genotypic relationship of the characters differed from the phenotypic one, although the sample size of the present study was too small to detect any statistical significance for the genetic parameters in some cases. The estimate of the genetic correlation of larval period on the two aphid species was positive and close to

Table 4 Jack-knife estimates for variance components (×100) of analyses in Table 3 and for broad sense heritabilities calculated using the variance component. Variance components were estimated using TYPE1 with host and sex as fixed effects. The parameters were jackknifed with each family omitted once so that the total number of iterations was equal to the number of families. Standard errors are given in parentheses

	Larval period			Pupal mass		
	A. pisum	*A. craccivora*	Artificial diet	*A. pisum*	*A. craccivora*	Artificial diet
Among-family	2.183 (1.115)	1.233 (0.567)	1.640 (0.930)	0.121 (0.176)	0.167 (0.335)	1.625 (0.663)
Within-family	0.667 (0.232)	2.697 (0.500)	1.722 (0.312)	2.140 (0.839)	2.886 (0.506)	3.920 (0.666)
Heritability	1.587 (0.208)	0.640 (0.250)	1.068 (0.377)	0.049 (0.237)	0.133 (0.231)	0.596 (0.199)

Table 5 Genetic correlation estimates of *Harmonia axyridis* between the same traits across different resource environments with their 95% confidence limits in parentheses

	A. pisum–*A. craccivora*	*A. pisum*–artificial diet	*A. craccivora*–artificial diet
Larval period	0.540 (−0.221, 0.892)	−0.325 (−0.325, 0.071)	−0.123 (−0.509, 0.303)
Pupal mass	0.184 (−0.439, 0.688)	0.368 (−0.269, 0.781)	−0.021 (−0.782, 0.622)

statistical significance, despite the large difference in the average values between the two aphid species. On the other hand, the genetic correlations between developmental traits on either of the two aphid species and that on the artificial diet were close to zero or negative. The discrepancy between the phenotypic and genetic relationship was also suggested by the significant genotype × resource interaction in the mixed ANOVAs and by the changes in the relative rank positions of family means in crossing reaction norms, when beetles raised on the aphids were compared with their siblings on the artificial diet.

These results suggest that the developmental traits on the two aphid species are genetically correlated, while there are only weak or no genetic correlations between the developmental traits on the aphid species and that on the artificial diet. These findings imply that selection to improve the ability to exploit an aphid species is expected to entail positive effects on the analogous ability on the other aphid species, but little or no effects on the artificial diet. Thus, different genotypes can be expected to achieve a high developmental performance on aphid resources and on the artificial diet.

Because of its ability to reduce aphid density, the potential of *H. axyridis* as a bio-control agent has been discussed (Ferran et al., 1996). The establishment of a mass rearing system and quality control are important for any bio-control program. In the rearing system, the production of a large number of an effective agent in a short period of time is vital. Artificial diet has produced superior results in rearing many predaceous ladybirds, including the present species (Matsuka & Niijima, 1985), making it possible to keep a large number of beetles continuously over a long period. On the other hand, the requirement for a higher production efficiency would consciously or unconsciously result in artificial selection for a shorter development time under mass rearing conditions. With the present estimate of the genetic correlation between the larval period on artificial diet and that on live aphids, however, one may need to be cautious with mass rearing using artificial diet, as it might lead to the evolution of different genotypes than the wild populations.

A possible explanation for the observed patterns of genetic architecture is that they might result from a functional relatedness between traits responsible for the exploitation of the aphid species and the artificial diet. Genetic studies have suggested that genetic correlation tends to be positive among functionally related traits (Conner & Via, 1993). Despite the delayed developmental process of the siblings reared on *A. craccivora*, it is possible that various traits responsible for the exploitation of the aphid species share the same physiological pathways.

Alternatively, these patterns could result from the ecological characteristics of the resource use of the ladybird beetle. For a predatory species, the relative abundance of resources fluctuates in time and space, and in that situation, adopting polyphagy would be strongly favored by natural selection, especially during larval stages. Potential genetic trade-offs between the use of different resources could be ameliorated by increased coordination among the loci, and a negative genetic correlation would be unlikely to be expected under the strong selection for being polyphagous. Thus, the present results could reflect the effect of selection favoring the ability to use different aphid species that can lead to the build up of linkage disequilibrium among loci responsible for the use of different aphids.

The effects of past selection to use aphid resources were suggested by the results of the analyses of variance components. There was a larger among-family variance component for pupal mass on the artificial diet when compared with that on the two species of aphids, and the estimates of the heritability reflected the patterns in the variation component. This pattern is consistent with the idea that an absence of selection on the ability to use a novel artificial diet results in the maintenance of genetic variance (Stearns, 1992). Larger among-family variance components and heritabilities were also reported for the developmental period and body size of the beetles reared on an artificial diet when compared with their siblings reared on tobacco aphids (*Myzus persicae*) (Grill et al., 1997). For the larval period on *A. pisum*, on the other hand, the reduced within-family variance component resulted in a high heritability. This pattern might show that beetle larvae were well canalized during their development on *A. pisum*, being buffered against random environmental noise. As stabilizing selection or selection with a convex fitness function favors genotypes which minimize the phenotypic variance of traits (Lande, 1980; Houle, 1992), this result may also suggest past adaptation of *H. axyridis* to the use of *A. pisum*. Overall, these results suggest the importance of the effects of selection to constantly use particular aphid species on the genetic architecture of the beetle. Insight into the effect of a prey exploitation pattern on the genetic architecture in *H. axyridis*, however, would still need further studies.

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