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Seasonality and genetic architecture of development time and body size of the birch feeding sawfly *Priophorus pallipes*

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Summary

We tested, using the sawfly *Priophorus pallipes* feeding on leaves of mountain birch, whether the expression of genetic (co)variation of larval development time and body size can be altered by exposing larvae to diets with differential seasonal changes in quality. In nature, larvae feed mainly on mature leaves, but occasionally they are forced to consume senescing leaves. Sixty families were assayed on three experimentally simulated diets: mature leaves of high quality, senescing leaves of rapidly declining quality, and senesced leaves of low quality. The intuitively obvious positive phenotypic and genetic correlations between development time and final mass were observed when the larvae consumed leaves of stable high quality, but low and declining food quality prevented long-growing individuals and families from achieving high final mass, switching the correlations to close to zero or negative in these treatments. The amount of genetic variation for body size showed a non-linear change across the diet quality gradient, whereas genetic variation for development time increased with decreasing diet quality. The among-trait difference in the degree reaction norms crossed along the diet gradient caused the changes in the expression of genetic (co)variation within the environments. Our results show that seasonally varying diet quality induces dramatic changes in the genetic (co)variation of development time and body size, and that simultaneous analysis of reaction norms and environment-specific expression of genetic (co)variation is necessary for the understanding of the genetic characteristics underlying the construction of phenotypes in heterogeneous environments.

1. Introduction

Genetic architecture of a population can be defined as the population genetic properties determining trait values, their intercorrelations, and responses of traits to environment (Falconer & Mackay, 1996; Roff, 1997; Lynch & Walsh, 1998). Genetic architecture can be characterized by quantitative genetic parameters measured within environments (heritabilities, genetic variances and genetic correlations; reviews by Mousseau & Roff, 1987; Hoffmann & Parsons, 1991; Houle, 1992; Roff, 1996; Hoffmann & Merilä, 1999; Roff & Mousseau, 1999) as well as by measures of phenotypic plasticity, i.e. the ability of a genotype to produce different phenotypes in different environments, and its genetic variation (Via & Lande, 1985; Schlichting, 1986; Stearns, 1989; Newman, 1992; Scheiner, 1993; Via *et al.*, 1995).

The amount of genetic variation together with the sign and degree of genetic covariation between traits dictate the rate, the magnitude and the direction of both direct and correlated evolutionary responses of traits to selection (Falconer & Mackay, 1996). Long-term predictive power of the genetic parameters depends, however, on their constancy (or proportionality) in time and space (Turelli, 1988). Several studies have found that genetic variation and genetic correlations vary across environments (reviews by Hoffmann & Parsons, 1991; Stearns *et al.*, 1991; Hoffmann & Merilä, 1999), indicating that environment-specific evolutionary responses to the same selection pressures may occur.

One factor potentially modifying genetic (co)variation across environments is phenotypic plasticity

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(Stearns, 1989; de Jong, 1990; Stearns *et al*., 1991). Phenotypic plasticity can be described as a reaction norm, which is the response curve of a genotype to an environmental gradient (Woltereck, 1909). The model by de Jong (1990) hypothesizes that due to genetic variation in reaction norms, indicated by non-parallel reaction norms, the expression of genetic variation as well as the sign of the genetic correlation between two traits may change across environments. Non-parallel reaction norms do not, however, automatically lead to changes in the genetic parameters (de Jong, 1990; Stearns *et al*., 1991). For instance, when the development of two traits is strongly integrated (e.g. wing and thorax length describing body size), the reaction norms of these two traits for a given genotype tend to show similar patterns. Thus, despite a possible genetic variation in the reaction norms, genetic correlations between the traits within environments may remain similar. Consequently, it is necessary to study the underlying reaction norms to understand the environment-specific expression of genetic (co)variation.

In nature, many species encounter seasonally varying environmental conditions which may shape their life-histories. In particular, seasonality may set constraints for the evolution of duration of growth stage and body size, the two key life-history traits (Roff, 1980, 1992; Tauber *et al*., 1986; Stearns, 1992). These two traits are usually positively correlated, long development time resulting in large body size (Roff, 1992; Stearns, 1992). However, many exceptions have been reported (Newman, 1988; Gebhardt & Stearns, 1988; Nylin, 1992; Gotthard *et al*., 1994; Abrams *et al*., 1996; Klingenberg & Spence, 1997; Kause *et al*., 1999*b*; Higgins, 2000), though the reasons for the variation in the body size–development time relationship have remained unexplained. An experimental manipulation of the seasonality of environment combined with a quantitative genetic analysis of the genetic architecture of the traits within and across the environments provides a powerful tool to examine whether seasonality could be one potential explanation for the variation in the relationship between body size and development time.

In our previous studies, we examined the ways in which seasonal variation in the foliage quality of mountain birch (*Betula pubescens* ssp. *czerepanovii*) is related to the genetic (co)variation of larval development time and body size of seven seasonally separated folivorous insect species feeding on mountain birch (six sawflies: Hymenoptera, Symphyta; and a moth: *Epirrita autumnata*, Geometridae) (Kause *et al*., 1999*b*; Kause *et al*., 2001). Leaf quality of birch for insect herbivores declines profoundly during both leaf growth and senescence, but remains stable during mid-summer (Haukioja *et al*., 1978; Ayres & MacLean, 1987; Hanhimäki *et al*., 1995; Nordell & Karlsson, 1995; Nurmi *et al*., 1996; Kause *et al*., 1999*a*), imposing differential time constraints for insects with different seasonal distributions and consequent differences in seasonal changes in diet quality during their growth stage. We found that in the three species consuming mainly mature birch leaves of stable quality, i.e. without diet-imposed time constraints for development time, long development led to high body mass, as shown by the strongly positive genetic correlations between development time and final body mass. On the other hand, the correlation was close to zero or negative in the four species consuming leaves of rapidly declining quality during leaf maturation or senescence. A long development time thus resulted in a high body mass and a consequent fitness advantage (large females have high realized fecundity; Kause, 2000) only when there were no strong diet-imposed time constraints for prolonged development (Kause *et al*., 1999*b*; Kause *et al*., 2001).

In the present paper, we tested whether the differential genetic correlations observed for the insect species inhabiting different environments are fixed properties of the populations, or whether they would be, due to genotype-by-environment interactions, induced by seasonally varying diet quality. To test for this, we experimentally simulated three different seasonal diets (mature leaves of high and stable quality, senescing leaves of rapidly declining quality, and senesced leaves of low quality) for 60 families of the sawfly *Priophorus pallipes*. This species is a typical feeder on mature birch leaves, but larvae have occasionally been found on yellowing leaves too (A. Kause, personal observation). This split-family study design allowed us to examine the ways in which the expression of genetic variation and correlations within environments are connected with the reaction norms describing expression of the traits across environments.

2. Methods

The study was conducted at the station of the Kevo Subarctic Research Institute in northernmost Finland (70° N, 27° E), in 1998.

(i) *Study objects*

Our study species, *P. pallipes* Lepeletier, is haplodiploid: fertilized eggs develop into females and unfertilized eggs into males. It overwinters as a prepupa within a cocoon, adults emerge in early summer, and females oviposit eggs within growing leaves of mountain birch, *B. pubescens* ssp. *czerepanovii* (Orlova). Larvae are solitary leaf chewers and they consume mature mountain birch leaves from mid-July until late August. The mountain birch is one

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of a few tree species able to survive in northernmost Europe. In our study area, leaves flush in early June, grow for a month and are shed around mid-September.

(ii) *Experimental design*

To induce plasticity into larval development time and final mass in a split-family experimental design, three diet quality treatments were established (H, stable high; D, temporally declining; L, stable low). In the H treatment, larvae consumed green mature leaves of stable and considerably high quality. In the D treatment, larvae consumed green leaves for 5 days, then senescing leaves (half green, half yellow) for 8 days, and finally yellow leaves of low quality for the rest of the time. In the L treatment, only yellow low-quality leaves were given to larvae, which is, in contrast to the other treatments, unlikely to occur in nature. To simultaneously obtain green, senescing and yellow leaves, we induced leaf senescence to field-growing trees a month before it naturally occurs, by manipulating the photoperiodicity to which the foliage was exposed. Branches from the trees were covered with black permeable plastic bags for 12 hours a day. This treatment mimicked the light regime of the autumn, inducing leaf senescence. Some branches were left untreated, to allow green leaves from the same trees to be available.

The experimental larvae were all male progenies of 60 unmated females collected from the field. The experiment was started with just-moulted fourth-instar larvae (for the pre-experimental methods see Hanhimäki *et al*., 1995). About 30 larvae from each of the 60 families were randomly assigned into the three treatments (i.e. 10 larvae per treatment and per family). A total of 1786 larvae were used. The larvae were kept singly in 48 ml plastic vials and offered fresh leaves according to their respective treatment. The position of the vials was randomized in the growth chamber. The larvae were fed *ad libitum*, and leaves were renewed every 3 or 4 days. To study only the effects of leaf quality variation on the larvae, temperature (12 °C) and light conditions (22 h light, 2 h dark) were kept constant during the experiment, mimicking the natural conditions of late summer in Lapland. To determine the end of larval period, the larvae were checked once a day to observe the cessation of feeding and the emptying of the gut. At this stage, individual larvae were weighed to the nearest 0·1 mg. Development time was measured as the number of days from the beginning of the experiment to the cessation of feeding.

Survival was high in all treatments: 99 % (591/596) on the stable high-quality food, 98 % (585/596) on the declining-quality food and 87 % (514/594) on the stable low-quality food. The genetic correlations of survival of the families with development time and final mass were weak ($r_G < 0{\cdot}13$, $P > 0{\cdot}32$), and we assume that the lower survivorship observed in the L treatment did not introduce a bias into the analyses.

(iii) *Statistical methods*

Jackknife estimates of heritabilities, coefficients of genetic and residual variation, phenotypic, environmental and genetic correlations were calculated within each treatment. In the GLM model (SAS Institute, 1990), the phenotypic (co)variance within each treatment was partitioned into (co)variation caused by among-family differences and by within-family differences (see tables 2.3 and 3.3 in Roff, 1997). Heritability in the broad sense was estimated as $h^2 = V_G/V_P = 2\sigma^2_G/\sigma^2_P$, where V_G and σ^2_G depict the causal and observational variance components of the among-family effect, and V_P and σ^2_P depict the respective components for total phenotypic variation. Development time was $\log_e$-transformed to normalize the distribution of the data. As heritability is a ratio, low heritability can result either from low genetic variation or from high residual variation (or both). Thus we calculated jackknife estimates of coefficients of genetic variation (CV_G) and residual variation (CV_R) as $CV_G = 100\ (V_G)^{1/2}/\text{population mean}$ and $CV_R = 100\ (V_P - V_G)^{1/2}/\text{population mean}$ (Houle, 1992). In contrast to heritabilities and correlations, *CV*s were calculated from the non-transformed data because they describe the true degree of variation when natural selection is of interest (Houle, 1992). Phenotypic, genetic and environmental correlations within the treatments were calculated as $COV_{XY}/(\sigma^2_X\sigma^2_Y)^{1/2}$ where COV_{XY} depicts covariance (phenotypic, genetic and environmental component) between the two traits and σ^2 depicts the respective variance components. In haplodiploid males, broad-sense estimates of heritabilities, coefficients of genetic variation and genetic correlations calculated using all-male families include additive genetic and maternal effects, but not dominance effects. A half-sib analysis of *P. pallipes* has shown that the broad-sense genetic correlation between development time and body mass and the broad-sense heritability of final mass are weakly influenced by maternal effects, whereas the broad-sense heritability of development time is inflated by maternal effects (Kause *et al*., 2001).

To examine the effect of the diet treatments on larval performance, mixed model analyses of variance with family (random), treatment (fixed) and family-by-treatment interaction (random) were performed (procedure GLM, SAS Institute, 1990, and the SAS approach described by Fry, 1992). To examine the degree to which the expressions of a trait measured in two different environments are genetically correlated, cross-treatment genetic correlations were calculated for each trait (Falconer, 1952; Via & Lande, 1985;

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Windig, 1997). First, jackknife estimates of the cross-treatment correlations were calculated from the (co)variance components as described by Fry (1992) for a mixed model SAS approach with unequal genetic variances in different environments. A jackknife procedure provides a straightforward way to test whether a correlation coefficient is significantly different from one, but it is a conservative method, especially when correlations are high (Windig, 1997). Second, Spearman rank correlations were calculated from the family means to examine the degree to which the ranking of families is changed across the treatments. In contrast to a correlation calculated from (co)variance components, a Spearman correlation is based only on the ranking of the families and no information on the magnitude of the differences between the families is utilized. Spearman correlations were not jackknifed, and it should be noted that the family means are biased (Fry, 1992). Thus these correlations are only indicative.

The jackknife procedure was performed by deleting one family each time after which the genetic parameters were recalculated. Then pseudovalues ($\hat{\theta}_i$) were calculated as $\hat{\theta}_i = n\hat{\theta}_n^0 - (n-1)\hat{\theta}_{n-1}^i$, where n denotes the number of pseudovalues (i.e. families), $\hat{\theta}_n^0$ the original estimate, and $\hat{\theta}_{n-1}^i$ the estimate with the family i left out. A jackknife estimate of a genetic parameter is a mean of all pseudovalues, which can be further used as replicates of the parameters to calculate standard errors and statistical tests (Knapp *et al.*, 1989; Roff & Preziosi, 1994; Roff, 1997). Significant deviations of heritabilities (one-tailed test), and correlations from zero (two-tailed test) and unity (one-tailed test), were tested using one-sample t-test. Differences among the treatments in heritabilities, CVs, and correlations were tested by performing one-way ANOVAs on the pseudovalues (Knapp *et al.*, 1989; Roff, 1997).

3. Results

The three diet treatments induced significant changes in the means of development time ($F = 623{\cdot}5$, $df_1 = 2$, $df_2 = 119{\cdot}07$, $P < 0{\cdot}0001$) and final larval mass ($F = 1609$, $df_1 = 2$, $df_2 = 118{\cdot}95$, $P < 0{\cdot}0001$): development time was the longest and final mass the lowest in the L treatment, while the opposite was true for the H treatment (Fig. 1). The values of the D treatment were intermediate. These results confirm that senescing and yellow leaves are of lower quality than green leaves.

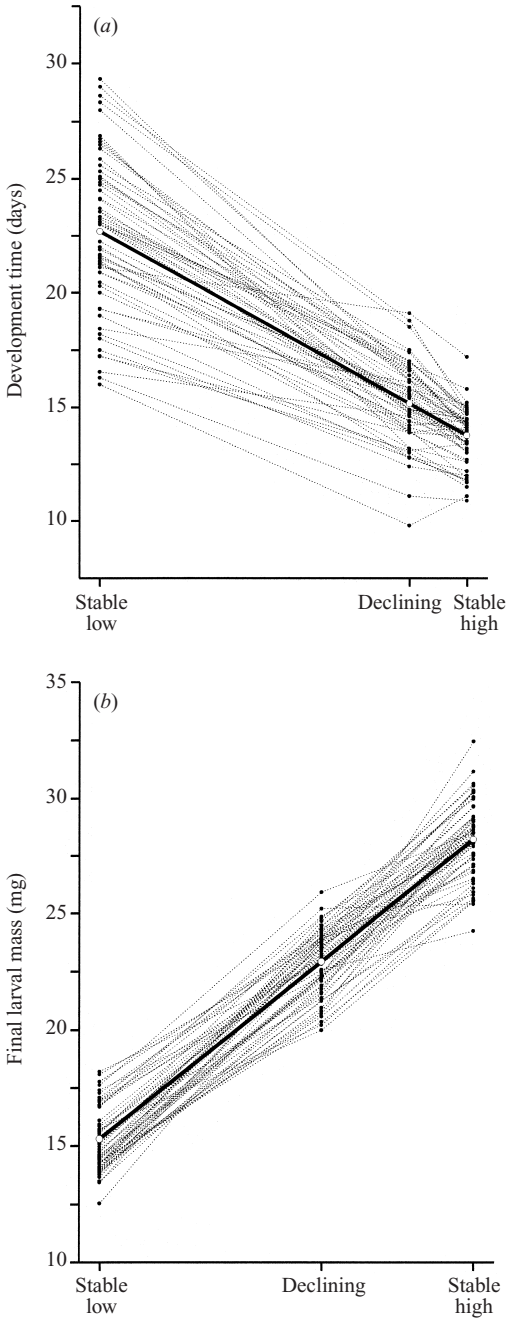

Fig. 1. Univariate reaction norms for (*a*) development time and (*b*) final larval mass. Each dotted line connects values of a given family across the three treatments. Population mean is shown by a dark line and white dots ($n = 60$ families). To facilitate the presentation, the population means on the three environments have been aligned.

(i) *Expression of genetic (co)variation within environments*

The diet quality treatments caused changes in the sign of the genetic and phenotypic correlations between larval development time and final mass calculated within the treatments (Table 1, Fig. 2). When the larvae consumed H leaves, the phenotypic and genetic correlations were strongly positive: the longer the larvae developed, the heavier they became. This result is consistent with our previous half-sib analysis with *P. pallipes* (Kause *et al.*, 2001). In the D

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Table 1. *Phenotypic* (r_P) *and environmental* (r_E) *correlations between larval development time and final mass measured in the three different diet quality treatments* (n = *60 families*)

Treatment	$r_P \pm$SE	$r_E \pm$SE
Stable high	0·29 ± 0·08***	−0·05 ± 0·14 NS
Declining	−0·45 ± 0·05***	−0·59 ± 0·08***
Stable low	−0·49 ± 0·03***	−0·47 ± 0·07***

NS, not significantly different from zero; ***$P < 0·001$.

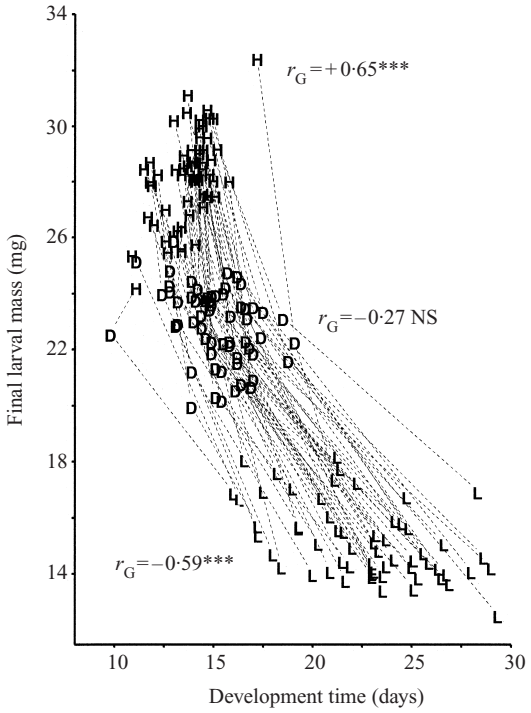

Fig. 2. Bivariate reaction norms of larval development time and final mass across the three diet quality treatments (H, stable high; D, declining; L, stable low). Each letter represents the position of the mean value for a given family, and the values of each family are connected with a line. Genetic correlations (r_G) within each treatment are given. $n = 60$ families.

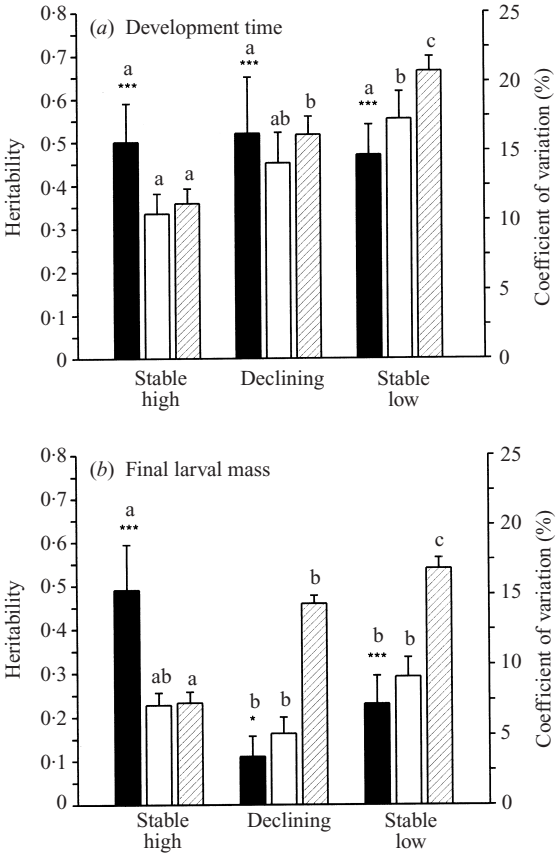

Fig. 3. Heritabilities ± SE (black bars), coefficients of genetic variation (white bars) and coefficients of residual variation (hatched bars) for larval development time (*a*) and final larval mass (*b*) in the three diet quality treatments. $n = 60$ families. *$P < 0·05$; ***$P < 0·001$. The bars marked with the same letter do not differ significantly at $P < 0·05$ level (contrast of one-way ANOVA for each parameter separately).

treatment, the genetic correlation was not significantly different from zero, and on the L diet, the phenotypic and genetic correlations were strongly negative. On the leaves of declining or stable low quality, long-growing individuals and families seemed to suffer and became smaller compared with the ones with short development time. The statistically significant change in the environmental correlation from near zero in the H treatment to strongly negative in the two other treatments (Table 1; $F_{2,177} = 7·68$, $P = 0·0006$) reflects further the deleterious environmental effects of declining and stable low leaf quality on the long-growing larvae.

In contrast to the heritabilities of development time ($F_{2,177} = 0·06$, $P = 0·94$), the heritability estimates of final mass ($F_{2,177} = 6·69$, $P = 0·0016$) and all coefficients of genetic and residual variation (range in $F_{2,177} = 3·07$–$47·82$ and in $P = 0·0488$–$0·0001$) differed significantly among the treatments (Fig. 3). Heritability of development time remained high and nearly constant across the treatments because both genetic and residual variation, measured as coefficients of variation, increased when the diet quality decreased. In other words, *CV*s of development time were higher on the L diet compared with the H diet (Fig. 3*a*). A different pattern appeared for the final larval mass. The heritability estimates and coefficients of genetic variation showed that genetic variation was the lowest in the D treatment (Fig. 3*b*), i.e. in the treatment where the genetic correlation was close to zero (Fig. 2). The heritability of final mass in the D treatment was significantly lower than in the H treatment, while the two-fold difference between the L and the D treatments was non-significant. CV_G of final mass was

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Table 2. *Cross-treatment genetic correlations* ($\pm SE$) *calculated according to Fry* (*1992*) *between the three treatments* (*H*, *stable high*; *D*, *declining*; *L*, *stable low quality*) *for development time and final larval mass*

Trait	H vs D treatments	D vs L treatments	H vs L treatments
Development time	$1{\cdot}04 \pm 0{\cdot}03$***	$0{\cdot}91 \pm 0{\cdot}10$***	$0{\cdot}81 \pm 0{\cdot}14$***
Final larval mass	$0{\cdot}55 \pm 0{\cdot}21$*	$0{\cdot}73 \pm 0{\cdot}21$***	$0{\cdot}43 \pm 0{\cdot}23$ NS

NS, not significantly different from zero; *$P < 0{\cdot}05$; ***$P < 0{\cdot}001$.

significantly lower in the D treatment compared with the L treatment, but there were no significant difference between the H and the D treatments (Fig. 3*b*).

(ii) *Expression of genetic constraints across environments*

The cross-treatment genetic correlations, calculated according to Fry (1992), were strongly positive and significantly different from zero for development time (Table 2), but they did not differ significantly from one (all P values $> 0{\cdot}08$). For final mass, the cross-treatment genetic correlations were weaker than those for development time. The correlations of final mass between the H and D treatments and the H and L treatments differed significantly from one ($P < 0{\cdot}02$), while the correlation between the D and L treatments did not ($P = 0{\cdot}10$). The Spearman rank correlations ranged from 0·56 to 0·75 (all P values $< 0{\cdot}0001$) for development time and from 0·24 to 0·30 (P values 0·02–0·06) for final mass. All in all, the cross-treatment genetic correlations show that across the treatments, the families reversed ranking more in the case of final larval mass than in the case of development time (see also Fig. 1). This among-trait difference in the strength of the genotype-by-environment interaction caused the changes in the expression of genetic (co)variation within the environments, as suggested by Stearns (1989), de Jong (1990) and Stearns *et al.* (1991).

4. Discussion

Using the sawfly *Priophorus pallipes* feeding on the foliage of mountain birch, we analysed genetics of larval development time and final body mass within and across diet treatments with differential seasonal changes in quality. In nature, the larvae of *P. pallipes* feed mainly on mature leaves, but in years with exceptional weather conditions they are forced to feed also on senescing leaves (A. Kause, personal observation). We demonstrated here that the two traits displayed different patterns in the genotype-by-environment interactions, as indicated by their different cross-treatment genetic correlations, generating dramatic modifications in the genetic variation and genetic correlations within the environments. Moreover, the low diet quality increased residual variation of the traits, altering heritability values. The effect of genotype-by-environment interactions on genetic (co)variation within environments has been modelled previously by Stearns (1989), de Jong (1990) and Stearns *et al.* (1991), and here we provide experimental support for their model.

(i) *Expression of correlations*

We found that on mature leaves of high quality long development time was genetically correlated with high body mass, but phenotypic, environmental and genetic correlations were switched to non-significant on leaves simulating rapid leaf senescence, and to significantly negative on senesced leaves (Table 1, Fig. 2). On the other hand, the comparison of seven seasonally separated insect species, including *P. pallipes*, feeding on the foliage of mountain birch showed that the genetic correlation between development time and body size is positive in the species feeding mainly on mature leaves of stable quality, whereas the species feeding mainly on growing or senescing leaves of declining quality display zero or negative correlations (Kause *et al.*, 1999*b*; Kause *et al.*, 2001). The present analysis provides evidence that the among-species difference in the genetic correlation may be caused by genotype-by-environment interactions. Other studies have also shown that a change in resource levels or environmental conditions may affect the magnitude and sign of genetic correlations between traits (e.g. Service & Rose, 1985; Gebhardt & Stearns, 1988; Stearns *et al.*, 1991; Simons & Roff, 1996).

Many models describing the evolution of body size assume a positive relationship between development time and final body size (see Roff, 1992; Stearns, 1992). Our results show that the growth trajectories of *P. pallipes* larvae were altered such that long development time led to large size only on the stable high-quality diet. Similar changes in the genetic development time–body size relationship have been observed for *Drosophila* on high- and low-quality food (Gebhardt & Stearns, 1988) and for spadefoot

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toads in ponds of long and short duration (Newman, 1988). Variability of development time–body size relationships is also predicted by the novel models of body size evolution which take into account the fact that individuals may change their growth rate according to environmental conditions (Rowe & Ludwig, 1991; Abrams *et al*., 1996). Since organisms tend to consume resources that they are adapted to, high environment quality may be commonly encountered by organisms, allowing the expression of the positive relationship between development time and body size in nature. Yet, seasonally varying food quality is encountered by many species. For instance, varying resource quality is encountered by many animals feeding on rotting fruits and fungi, on drying dung, on decaying corpses, by amphibians inhabiting temporary ponds, by plants growing on soils of seasonally varying nutritional quality, and by pioneer species invading new habitats with increasing level of competition (Begon *et al*., 1996). Moreover, photoperiod has been shown to influence the relationship between development time and body size (Tauber *et al*., 1986). Accordingly, non-zero and negative relationships between development time and final size can be expected to appear in nature, as shown for the insect guild feeding on mountain birch (Kause *et al*., 1999*b*; Kause *et al*., 2001) and for other animals (Newman, 1988; Nylin, 1992; Gotthard *et al*., 1994; Klingenberg & Spence, 1997; Higgins, 2000).

(ii) *Expression of genetic variation*

The model by de Jong (1990) and Stearns *et al*. (1991) suggests that the degree of genetic variation of a trait can be minimal in the environment where a sign of genetic covariation between this and another trait changes in sign. Our results indeed show that genetic variation for final larval mass, measured as heritability and coefficient of genetic variation, was the lowest in the treatment with declining diet quality (Fig. 3). Accordingly, in this treatment, genetic correlation between larval development time and final mass was close to zero and the sign was changed at the opposite extremes of this treatment (Fig. 2). In contrast to final mass, heritability of larval development time remained unaltered in all three treatments. The analysis of the coefficients of variation showed that the genetic variation for development time in fact increased as a function of decreasing diet quality (Fig. 3). Recent reviews suggest that the amount of genetic variation often increases when organisms are exposed to stressful environments, but a large body of studies disclose the opposite (Hoffmann & Parsons, 1991; Hoffmann & Merilä, 1999). As hypothesized by the model (de Jong, 1990; Stearns *et al*., 1991), our results suggest that non-linear trends in the expression of genetic variation along an environment gradient may occur, at least in some traits (body mass in our data). The non-linearity, which can be observed only by using experiments with more than two environments, could potentially provide one explanation for the inconsistency in studies examining the relationship between the level of environmental stress and the amount of genetic variation expressed within environments.

The ultimate reason(s) for the changes in genetic (co)variation across environments can be manifold (de Jong, 1990; Stearns *et al*., 1991; Hoffmann & Merilä, 1999). For instance, one may hypothesize that we should find, as a result of strong selection, antagonistic genetic correlations on mature leaves (i.e. a positive correlation in the case of development time and body size), and zero or negative correlations in the environments not commonly encountered. This is what was observed. Similarly, it can be argued that genetic variation should be low in the environment most commonly encountered, while the variation should be high in rare and unfavourable conditions (for reasons why see Hoffmann & Merilä, 1999). This hypothesis is supported by the CV_Gs of development time, but genetic variation for final mass showed a non-linear trend and was the lowest on rapidly senescing leaves (Fig. 3). The current data provide no clear evidence why the genetic (co)variation is changed across the environments in *P. pallipes*, but understanding the way reaction norms evolve would help us to solve this problem (de Jong, 1990).

We observed high amounts of genetic variation for both development time and final larval mass of *P. pallipes* on mature leaves, on their most commonly encountered diet. In an accompanying paper (Kause *et al*., 2001), we show that the insect species feeding on mountain birch during early summer show significantly lower heritabilities and CV_Gs for development time than the mid-summer species such as *P. pallipes*, whereas the autumn species exhibit the highest heritabilities and CV_Gs. Leaf growth and the associated decline in the suitability of leaves for herbivores (Haukioja *et al*., 1978; Ayres & MacLean, 1987; Hanhimäki *et al*., 1995; Nordell & Karlsson, 1995; Nurmi *et al*., 1996; Kause *et al*., 1999*a*) impose strong phenotypic selection for rapid larval development during early summer (Ayres & MacLean, 1987), and we have hypothesized that this may have eroded genetic variation from the duration of development time in the early-season birch folivores (Kause *et al*., 1999*b*, 2001). This strong selection pressure is relaxed after leaf maturation. Moreover, considerable annual variation in the onset of autumn during early and late autumns (Kause *et al*., 2001) may promote genotype-by-environment interactions and may result in an annual variation in the strength of selection on development time, maintaining high amounts of genetic variation in the development time

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of *P. pallipes* and the autumn species. When different herbivore species are compared, there is no seasonal trend in the amount of genetic variation for body size, suggesting that seasonally occurring factors are not moulding the expression of genetic variation in final size (Kause *et al*., 2001).

(iii) *Determination of plastic responses*

Phenotypic plasticity can be either adaptive or non-adaptive. In nature, when food quality becomes low due to leaf senescence, the time remaining for *P. pallipes* larvae to complete their development is reduced. Early winter frosts have been observed to kill the late-developing sawfly larvae feeding on senescing leaves (P. Niemelä & L. Iso-Iivari, personal communication 1999). We observed that the larvae prolonged their development time in response to leaf senescence (Fig. 2), and the risk of death thus seems to become higher, not lower. Furthermore, assuming that females perform similarly to males, a reduction in body mass on senescing leaves is accompanied by decreased fecundity (large females have high realized fecundity; Kause, 2000). In contrast to these potential costs, it might be objected that development time is prolonged in response to lower growth rate on low-quality diets to gain mass and a consequent fitness advantage (Stearns & Koella, 1986). For instance, a minimum size may be required to produce eggs and/or sperm, giving larvae no choice but to extend their development time. Moreover, selection on the shape of the reaction norm on senescing leaves may be very weak, because the slowly growing larvae that encounter senescing leaves may contribute little to the future gene pool in the field, and thus an optimal reaction norm never evolves (Kawecki & Stearns, 1993). Consequently, it is not possible to conclude whether the observed plasticity is adaptive or non-adaptive, and a detailed analysis should be conducted to reveal the fitness consequences of different plastic responses in *P. pallipes*. However, we are able to conclude that the declining leaf quality during the end of the growing season is not used as an environmental cue by the larvae to shorten larval development time. Interestingly, other studies have shown that pond drying in spadefoot toads (Newman, 1988, 1992), limited resources in dung flies (Blanckenhorn, 1998) and photoperiod in insects (Tauber *et al*., 1986) shorten larval development times. Photoperiod may also be used by sawflies to adjust the termination of development time in nature.

(iv) *Environment-dependent evolutionary responses*

Environment-specific expression of genetic correlations and variation has several evolutionary implications. Instability of genetic parameters does not allow us to predict precisely the evolutionary response of a population to phenotypic selection (Turelli, 1988). Genetic correlations and the lack of genetic variation in traits constrain the possible set of life-history strategies that can evolve in a population (Falconer & Mackay, 1996). Some of these constraints may be eliminated in specific environments, revealing new adaptive life-history solutions previously constrained by the genetic architecture. On the contrary, new constraints not previously encountered by a population may also appear. Our data show that the amount of genetic variation of final mass was significantly reduced on the temporally declining leaf quality. In addition to seasonality, host plant shifts of herbivores can be accompanied by changes in diet quality (Slansky & Rodriguez, 1987) which may modify genetic parameters of a population. Consequently, even if phenotypic selection pressures are similar between the host plants, the population means will evolve to different values at a different rate. In addition to direct effects of phenotypic selection on traits, the change in the sign of a genetic correlation will alter the direction of correlated responses to phenotypic selection (Falconer & Mackay, 1996). The results of this study show that simultaneous analysis of reaction norms and environment-specific expression of genetic (co)variation helps our understanding of the genetic characteristics underlying the construction of phenotypes in heterogeneous environments.

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