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EFFECTS OF TEMPERATURE AND DIETARY NITROGEN ON GENETIC VARIATION AND COVARIATION IN GYPSY MOTH LARVAL PERFORMANCE TRAITS

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Abstract - To assess the plastic and genetic components of variation in responses of gypsy moth (*Lymantria dispar*) 4th instar larvae to temperature and food quality, we applied a split-family four-environment experimental design where full-sibs were reared on two constant temperatures (23°C and 28°C) and two concentrations of dietary nitrogen (1.5 and 3.7% dry weight). A temperature of 28°C and low dietary nitrogen decreased larval weight and prolonged larval developmental time, while viability was not affected. Only a marginally significant interaction between the two environmental factors was found for larval weight. The broad-sense heritability for larval developmental time did not change across environments, and across-environment genetic correlations were close to one. Heritability for larval weight depended on environmental and across-environmental genetic correlations that were not significant. There was no evidence of a trade-off between developmental time and larval weight. The implications of the obtained results for the evolution of phenotypic plasticity in complex environments are discussed.

Key words: Phenotypic plasticity, temperature, nitrogen, heritability, genetic correlations, larval performance, *Lymantria dispar, Serbia*

INTRODUCTION

Temperature and food quality are the most important determinants of insect performance. Stressful temperature, low and/or imbalanced nutrient content and the presence of toxic allelochemicals in food reduce the survival, rate of development, body size and reproduction of herbivorous insects (Simpson et al., 2009; Fischer and Karl, 2010). Among nutrients, protein (nitrogen) content has the most profound effect (Mattson, 1980). The successful growth of organisms exposed to spatial and temporal variation in these environmental factors relies on their capability to phenotypically match environmental needs.

Phenotypic plasticity, as the ability of a genotype to produce different phenotypes depending on environmental conditions, is central for understanding an organism's evolution in heterogeneous environments (West Eberhard, 1989). Empirical studies of phenotypic plasticity quantify genotype-by-environment interactions, trait heritabilities within environments and across-environment genetic correlations. The existence of genetic variation in the ability of organisms to cope with different environments has implications for their evolution towards specialization or increased phenotypic plasticity. Selection can change the reaction norm by acting not only on variation in the reaction norm but also on a trait variation within specific environments (Via et al., 1995; Schlichting and Pigliucci, 1995). Across-environment genetic correlations close to one represent constraints for the evolution of optimal phenotypic plasticity (Scheiner, 1993).

There is a significant level of genetic variation in the relative performance of insects at different temperatures (Brakefield and Kesbeke, 1997; Kingsolver

et al., 2004; Bentz et al., 2011) and on different host plants (Via, 1984; Lazarević et al., 1998; Ueno et al., 2001). Significant genotype-by-environment interaction has also been revealed in experiments with artificial diets that differ in nutrient and allelochemical content (Mrdaković et al., 2011; Savić et al., 2011).

However, as pointed out by Stillwell et al. (2007), organisms live in an ecologically complex world that necessitates experiments with a simultaneous variation of multiple environmental factors. Many studies have confirmed the significant interactive effects of temperature and food quality on insect performance (Stamp and Horwath, 1992; Lindroth et al., 1997; Petersen et al., 2000; Levesque et al., 2002). This interaction may affect genetic variance within an environment as well as genetic covariance across environments (Kingsolver et al., 2006).

The present study deals with separate and interactive effects of rearing temperature and dietary nitrogen on the survival, developmental time and weight of 4th instar gypsy moth (*Lymantria dispar* L.) larvae. To contribute toward understanding the genetic basis of phenotypic plasticity, we explored how trait heritabilities and across-environment genetic correlations changed depending on the environment. The highly polyphagous feeding nature, outbreaking population dynamics and wide distribution in the forests of the northern hemisphere make the gypsy moth a suitable model system for studying phenotypic plasticity. A genetic variation of phenotypic plasticity in response to host plants has been reported for the gypsy moth (Rossiter, 1987; Lazarević et al., 1998; Lazarević et al., 2002). However, to the best of our knowledge, the results presented in this paper are the first report on a quantitative genetic analysis of the thermal sensitivity of gypsy moth performance.

MATERIALS AND METHODS

Rearing conditions and measuring larval performance traits

Gypsy moth egg masses were collected from a poplar forest in Opovo (40 km north of Belgrade, Serbia). They were surface-sterilized in 0.1% sodium hypochlorite and set out for hatching. Since female gypsy moths do not remate except in dense populations (Carde and Hagaman, 1984), larvae hatched from a single egg mass represent full-sibs. Larvae were reared in Petri dishes at a 12 h light:12 h dark photoperiod at four combinations of temperature and dietary nitrogen. We used a split full-sib family experimental design with two different temperatures (23°C and 28°C) and two different concentrations of dietary nitrogen (1.5% and 3.7% dry weight). Artificial diets were modified from a high wheat germ diet (O'Dell et al., 1985) according to Lindroth et al. (1997).

We determined the following performance traits in 4th instar gypsy moth larvae: viability, developmental time and weight. Mortality and molting were recorded daily. The viability was determined by a daily count of dead individuals and calculation of the percentage of larvae that molted into the 4th instar. The developmental time was the period between hatching and molting into the 4th instar, and larval weight was measured at the beginning of the 4th instar.

Data analyses

The significance of the main and interactive effects of temperature and dietary nitrogen content were evaluated by two-way analysis of variance (ANOVA). Specific post-hoc comparisons were carried out by Scheffe's test. Three-way linear mixed-effect ANOVA with temperature and food as fixed effects and family (egg-mass) as a random effect was used to test for the significance of genetic variation in phenotypic plasticity in response to temperature and nutritive stress. Due to poor hatching in some of the egg masses, only 15 out of 19 egg masses were included in 3-way ANOVA. Appropriate ANOVA models were applied to arcsin square-root transformed values of viability and log-transformed values of developmental time and larval weight.

Broad-sense heritabilities (h^2) were calculated according to standard formulae for the unbalanced

full-sib design (Becker, 1984). Genetic correlations within and across environments were calculated as Pearson's product-moment correlations of family means. A Z-test was applied for comparison of heritabilities and genetic correlations between the treatments (Sokal and Rohlf, 1981).

RESULTS AND DISCUSSION

Similar to the results obtained for other lepidopteran species (Broadway and Duffey, 1986; Woods, 1999; Levesque et al., 2002; Kingsolver et al., 2004), our results showed that stressful temperature and low nitrogen concentration combined with an artificial diet decrease the performance of gypsy moth larvae. While larval viability was not affected by temperature ($F_{1,68} = 1.18$, $P = 0.281$) and food quality ($F_{1,68} = 0.14$, $P = 0.712$; Fig. 1A), both factors exhibited strong effect on developmental time and larval weight. On average, developmental time was significantly increased in response to low nitrogen concentration ($F_{1,309} = 11.89$, $P < 0.001$), whereas still faster development was recorded in larvae reared at 28°C ($F_{1,309} = 299.20$, $P < 0.001$; Fig. 1B). Fourth instar gypsy moth larvae were smaller if reared on a low nitrogen diet ($F_{1,309} = 21.71$, $P < 0.001$) and higher temperature ($F_{1,309} = 132.09$, $P < 0.001$; Fig. 1C).

It has been shown that a low protein (nitrogen) diet provoked prolonged larval development and decreased pupal size when gypsy moths were reared on a low quality diet throughout larval development (Rossiter, 1987; Lindroth et al., 1997). Young larvae appeared to be more sensitive than 5th and 6th instar larvae (Stockhoff, 1992) and females were more sensitive to a lack of nitrogen than males, due to the allocation of resources toward synthesis of vitellogenin during the final larval instar (Lindroth et al., 1997). Food quality affects insect performance via its effects on food consumption and utilization. Although an adaptive increase in consumption rate has been recorded on host plants and artificial diets with low protein (nitrogen) content, the gross growth efficiency of the gypsy moth was generally decreased (Stockhoff, 1992; Lindroth et al., 1997; Lazarević et al., 2002).

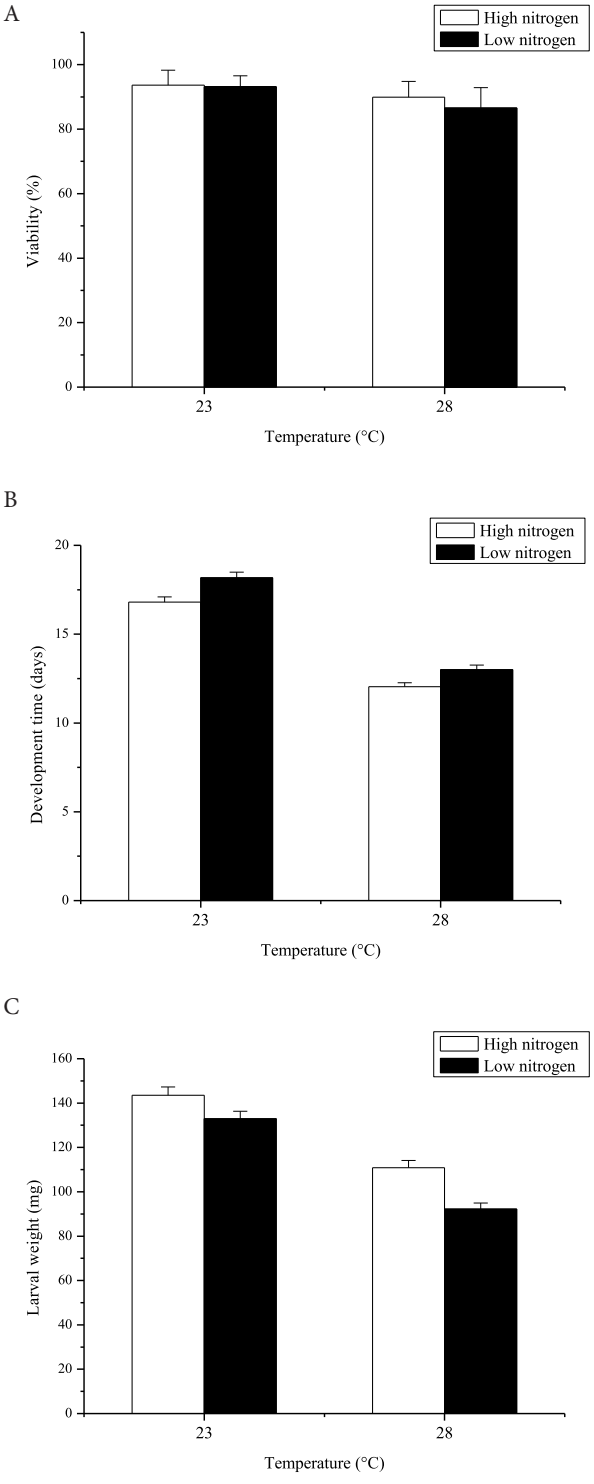

Fig. 1. Viability (A), developmental time (B) and larval weight (C) of 4th instar gypsy moth larvae in response to temperature and dietary nitrogen. Values are means and standard errors.

Table 1. Broad-sense heritabilities (h^2±SE) for developmental time and weight of 4th instar gypsy moth larvae depending on temperature and food quality. n – number of families; * P < 0.05; ** P < 0.01.

		Developmental time		Larval weight	
Temperature	Nitrogen content	h^2	±SE	h^2	±SE
23°C	High (n=18)	0.8330**	0.2442	-0.0600	0.1537
	Low (n=19)	0.4859	0.2380	0.5485*	0.2414
28°C	High (n=17)	0.5653*	0.2539	0.1422	0.2073
	Low (n=16)	0.5878*	0.2637	0.0348	0.1937

Table 2. Genetic correlations between developmental time and larval weight in 4th instar gypsy moth larvae reared on two constant temperatures and two concentrations of dietary nitrogen. * P < 0.05.

Environment		Temperature	
		23°C	28°C
Nitrogen	High	-0.1641	-0.5211*
	Low	-0.5563*	-0.5789*

Table 3. Across-environment genetic correlations for developmental time and weight of 4th instar gypsy moth larvae. * P < 0.05; ** P < 0.01.

Correlation		Developmental	Larval
Environment 1	Environment 2	time	weight
23°C, high nitrogen	23°C, low nitrogen	0.5553*	0.2140
28°C, high nitrogen	28°C, low nitrogen	0.7056**	0.4638
23°C, high nitrogen	28°C, high nitrogen	0.6744**	0.3024
23°C, low nitrogen	28°C, low nitrogen	0.6495**	0.4670

Investigations into temperature effects on gypsy moth performance are scarce. Maksimović (1958) has recorded lower larval duration, pupal weight and fecundity in gypsy moths reared at a constant temperature of 28°C than at 24°C. The gypsy moth growth rate has a plateau between 28°C and 32°C and developmental threshold at 12°C (Pantyukhov, 1962). Similar to our results, larval viability was not affected by temperature while a change of temperature regime from 19°C:16°C to 25°C:22°C shortened developmental time and increased the growth rate of 4th instar gypsy moth larvae (Lindroth et al., 1997).

Contrary to studies which found that the thermal reaction norms for the individual performance of herbivorous insect were affected by nutritive value of their food (Petersen et al., 2000; Levesque et al., 2002; Kingsolver et al., 2006), our study showed that temperature and dietary nitrogen influenced all three larval performance traits independently, i.e., the "temperature × nitrogen" interaction term in 2-way ANOVA was not significant. Only a marginally significant interaction was recorded in larval weight ($F_{1,309} = 3.45$, $P = 0.064$). Larvae reared at the stressful temperature of 28°C were more sensitive to low dietary nitrogen. Low nitrogen decreased larval weight

by approximately 8% at 23°C (P = 0.245, Scheffe's test), while a decrease of 17% was recorded at 28°C (P = 0.0002, Scheffe's test). Lindroth et al. (1997) also failed to find a significant "temperature × nitrogen" interaction for the larval survival, developmental time and pupal weight of the gypsy moth while the interaction was significant for some nutritional indices. The lack of interaction indicates the simple effect of multiple environments on gypsy moth performance, i.e., independence of reaction norms along different environmental axis.

Understanding the evolution of reaction norms in heterogeneous environments requires knowledge of the sensitivity of the genetic variation of a trait to environmental changes, the significance of genetic variation in trait plasticity, and the existence of trade-offs between environments.

Three-way ANOVA revealed a significant family effect on developmental time ($F_{14,214}$ = 4.34, P = 0.0103) while, on average, families did not differ significantly in larval weight ($F_{14,214}$ = 4.21, P = 0.2558). Data on broad-sense heritabilities for larval performance traits are summarized in Table 1. The values of heritabilities for developmental time were moderate to high and, with the exception of the 23°C/low nitrogen treatment, significantly different from zero. On the other hand, a significant expression of genetic variation for larval weight was recorded only with the 23°C/low nitrogen diet. This value was significantly higher than the heritability in larvae reared on a high nitrogen diet (t = 9.96, $P < 0.001$; z-test). Temperature exhibited a strong effect on the heritability of larval weight. The expression of genetic variation was slightly increased in response to 28°C at high nitrogen concentration (t = 2.79, $P < 0.05$; z-test), whereas a decrease in heritability was recorded at low nitrogen concentration (t = 2.39, $P < 0.05$; z-test). Phenotypic plasticity can modulate the expression of genetic variation by influencing the within- and/or among-family variance of a trait. Accordingly, unfavorable conditions can either increase or decrease the heritability (Hoffmann and Merilä, 1999). Our results showed that the increased heritability of larval weight in the 23°/low nitrogen environment was mostly the consequence of a genetic variance that was about twice as large as the genetic variance in 23°C/high nitrogen and 28°C/low nitrogen environments. Previous studies of the gypsy moth have not revealed significant changes in the heritability of pupal weight in response to the low protein content of an artificial diet (Rossiter, 1987), unsuitable host plant (Lazarević et al., 1998; Lazarević et al., 2002) or increased population density (Lazarević et al., 2008). Significant differences in trait heritabilities between environments might facilitate the evolution of phenotypic plasticity even when there is no significant genotype-by-environment interaction. In the gypsy moth population from a locust-tree forest, more than fifty generations of selection for adaptation to an unsuitable host plant characterized by a low protein and high allelochemical content, led to a depletion of genetic variation in the weight of 5th instar larvae reared on a high wheat germ diet and a significant increase of the plasticity in response to tannic acid (Mrdaković, 2010).

Neither of performance traits showed a significant "family × temperature" ($F_{14, 214}$ = 1.53, P = 0.217 for developmental time and $F_{14, 214}$ = 0.81, P = 0.653 for larval weight) or "family × nitrogen" interaction term ($F_{14, 214}$ = 1.58, P = 0.201 for developmental time and $F_{14, 214}$ = 0.69, P = 0.754 for larval weight), indicating that there was no significant variation in phenotypic plasticity. In other words, the gypsy moth is a true generalist since all families exhibited similar responses to changes in temperature and food quality. The lack of significant "family × food" interaction has also been demonstrated for the developmental time and pupal weight of gypsy moths reared on an artificial diet with a different protein content (Rossiter, 1987). In experiments with natural host plants, in the gypsy moth there is a significant variation in diet breadth (Rossiter, 1987; Lazarević et al., 1998; Lazarević et al., 2002). In other herbivorous insects, a significant variation in phenotypic plasticity in response to host plants (Ueno et al., 2001; Milanović and Gliksman, 2004) and temperature has been found (Gilchrist, 1996; Kingsolver et al., 2004; Bentz et al., 2011).

Beside trait variation and variation in trait plasticity, negative genetic correlations (trade-offs) across environments as well as among different performance traits within environments, are important for understanding the evolution of host plant and thermal specialization. Within-environment trade-offs contribute to the maintenance of genetic variation of life-history traits in natural populations and are a fundamental presumption of their evolution (Reznick, 1992). In the present study, we quantified genetic correlations between the developmental time and larval weight of the gypsy moth. Although larger individuals are expected to have a longer developmental time, such a trade-off has not been found in outbreaking Lepidoptera (Tammaru et al., 2000). Our results, presented in Table 2, showed that under the most suitable conditions (23°C, high nitrogen) there was no association between these traits. At a stressful temperature and low food quality we recorded significant negative correlations between the developmental time and larval weight. This correlation was not sensitive to environmental changes. Under stressful conditions, the strategy of the gypsy moth is the maximization of the growth rate. The positive genetic correlation between the developmental rate and body size suggests a faster fixation (elimination) of alleles with positive (negative) effects on the traits.

All genetic correlations across environments are positive (Table 3). The positive across-environment genetic correlations are characteristic for generalists and indicate that there are common genes that determine a trait in different environments. However, if positive correlations significantly differ from one, selection may favor specialization in heterogeneous environments (Fry, 1996). For the developmental time, correlations were mostly high and significant. As revealed by the z–test, their values were close to one, which represents a constraint for the evolution of optimal phenotypic plasticity. The evolution of phenotypic plasticity for developmental time is further hampered by a lack of significant genotype-by-environment interaction and a lack of significant differences in heritabilities between environments. The opposite was found for across-environment correlations for larval weight, i.e., all correlations were non-significant and significantly different from one. Although there was no variation in phenotypic plasticity, the evolution of specialization was possible due to significant differences in heritabilities for larval weight between environments. Across-temperature genetic correlations did not differ between the diets and, similarly, across-diet genetic correlations did not depend on temperature, pointing to the stability of the genetic architecture underlying phenotypic plasticity. Stillwell et al. (2009) also recorded significant positive across-temperature genetic correlations close to one for the performance traits of *Callosobruchus maculatus* that were not affected by the host plant. On the other hand, in *Pieris rapae* the across-temperature genetic correlation for growth rate changed from a high significant positive value on an artificial diet to a non-significant negative value on a natural host plant (Kingsolver et al., 2006).

Although we failed to find significant "temperature × food quality" interaction, it is more likely that in natural conditions the interaction of many biotic and abiotic factors shapes the complex reaction norms of gypsy moth performance traits. For example, temperature affects the susceptibility of the gypsy moth to pathogens (van Frankenhuyzen et al., 2008) and the suitability of gypsy moth host plants varies with temperature (Williams et al., 2000), CO2 (Traw et al., 1996), altitude (Erelli et al., 1998), defoliation (Rossiter et al., 1988), sunspot activity (Selås et al., 2004; Milenković et al., 2010), etc. Studies on the adaptive potential of herbivorous insects to respond to complex environments are relevant for understanding host range and thermal sensitivity evolution as well as for predicting population dynamics and alterations in species distribution due to climate change.

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