TESTING THE ADAPTIVE PLASTICITY OF GYPSY MOTH DIGESTIVE ENZYMES IN RESPONSE TO TANNIC ACID USING PHENOTYPIC SELECTION ANALYSIS

Marija MRDAKOVIĆ[1*], Biljana STOJKOVIĆ[2, 3], Larisa ILIJIN[1], Milena VLAHOVIĆ[1], Vesna PERIĆ-MATARUGA[1], Jelica LAZAREVIĆ[1]

[1]Department of Insect Physiology and Biochemistry, Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia
[2]Department of Evolutionary Biology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia
[3]Faculty of Biology, University of Belgrade, Belgrade, Serbia

Mrdaković M., .B. Stojković, L. Ilijin, M. Vlahović, V. Perić-Mataruga, and J. Lazarević (2014*): Testing the adaptive plasticity of gypsy moth digestive enzymes in response to tannic acid using phenotypic selection analysis* - Genetika, Vol 46, No. 3, 883-894

The adaptive significance of plasticity of digestive enzyme responses to allelochemical stress was tested on 32 full-sib gypsy moth families from an oak forest (the *Quercus* population) and 26 families from a locust-tree forest (the *Robinia* population), reared on control or tannic acid-supplemented diets. By using the relative growth rate as a fitness measure in phenotypic selection analyses, we revealed that higher specific activity of leucine aminopeptidase in *Quercus* larvae and lower specific activity of trypsin in *Robinia* larvae were adaptive in the control environment. In *Quercus* larvae, elevated specific activities of leucine aminopeptidase and lipase were adaptive in the stressful environment. There were no plasticity costs for the enzyme activities in either experimental group. The obtained results suggest that adaptive plasticity of digestive enzyme activity in gypsy moth larvae contributes to optimal growth rate under various environmental conditions.

Key words: allelochemical stress, digestive enzymes, *Lymantria dispar* L., relative growth rate, selection gradients

INTRODUCTION

The gypsy moth is one of the most serious insect pests of the northern hemisphere. Considering the wide range of host plants (VANHANEN *et al.* 2007), gypsy moth larvae are exposed

_____

Corresponding author: Marija Mrdaković, Department of Insect Physiology and Biochemistry, Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia, email: mm1507@ibiss.bg.ac.rs

to various selective pressures imposed by numerous host-specific allelochemicals. Increased content of allelochemicals in leaves or in artificial diets significantly influence gypsy moth performance (BOURCHIER and NEALIS 1993; LAZAREVIĆ *et al*. 2002; HEMMING and LINDROTH 2000; BARBEHENN and KOCHMANSKI, 2013). In the previous study (MRDAKOVIĆ *et al*. 2011), we recorded significant negative effects of a tannin-supplemented artificial diet on masses of gypsy moth larvae originating from oak and locust-tree populations. For the gypsy moth, oak species are preferred hosts, while the locust-tree is one of the hosts that larvae avoid due to the high allelochemical content in leaves (BARBOSA and KRISCHIK 1987; LAZAREVIĆ *et al*. 2003). Long-term evolution of gypsy moth populations in locust-tree forest (SIDOR and JODAL 1983) has led to significant population divergence for various life-history, behavioral and physiological traits (LAZAREVIĆ 2000; LAZAREVIĆ *et al*. 2002, 2003; MRDAKOVIĆ 2010; MRDAKOVIĆ *et al*. 2013a). With regard to digestive enzymes, previously published results (MRDAKOVIĆ *et al*. 2013a, 2013b) revealed changes in the specific activity of digestive proteases, α-glucosidase and lipase in gypsy moth larvae from these populations, in response to allelochemical stress. Diverse responses of two local populations to tannin treatment were recorded for α-glucosidase and lipase activities. Significant expression of genetic variation in enzyme activities, as well as their plasticity, detected within both populations, points to the potential for evolution of an adaptive plastic response to stressful environments. Being that inter-relations between various life-history traits determine the evolution of life-history strategy and net relative fitness in a population within specific environment (STEARNS 1992), plastic responses of digestive enzymes could have various patterns of effects on different life-history traits. Recently, using phenotypic selection analyses where the duration of larval development was an indirect measure of fitness, we revealed adaptive plastic responses of listed digestive enzymes that contribute to gypsy moth fitness under allelochemical stress (MRDAKOVIĆ *et al*. 2014). In the present study, we present results on how changes in activity of gypsy moth digestive enzymes influence relative growth rate (RGR) - the parameter which is frequently used as a proxy of fitness value.

Insect growth rate is a plastic trait that can be affected by many environmental factors, such as photoperiod, temperature, density, dietary variations (STOCKHOFF 1992, 1993b; TAMMARU *et al*. 2000, 2004; MARGRAF *et al*. 2003; OJEDA-AVILA *et al*. 2003; KINGSOLVER *et al*. 2004; JANKOVIĆ-TOMANIĆ and LAZAREVIĆ 2012). In phytophagous insects larval growth encompasses several physiologically different instars (STOCKHOFF 1993a; NIJHOUT 1994) exposed, among others, to spatial and temporal variations in host plant chemical composition (HEMMING and LINDROTH 1995; SCHEIRS *et al.* 2002). Individuals that grow faster achieve larger pupal and adult masses during shorter juvenile development. For folivorous insects, this adaptive trait enables avoidance of feeding on low quality mature leaves during younger instars and shortens the period of exposure to parasites and pathogens. In addition, faster growth of these insects is accompanied with increase of body mass, which is positively correlated with reproductive potential. This is especially important for insects with a non-feeding adult stage, where reproductive potential depends on the resources accumulated in larval periods. As a fitness trait, growth rate itself can be subjected to natural selection (reviewed in NYLIN and GOTTHARD 1998). For example, higher growth rate can be favored in species with outbreaking population dynamics (TAMMARU *et al*. 2000). Differences in larval growth result from variation in underlying behavioral, physiological and biochemical characteristics (GOTTHARD 2008). Plastic adjustments of physiological responses allow phytophagous insects to cope with harmful effects of allelochemical stress and maintain functional strategies for survival and reproduction in heterogeneous environments. Rapid relative

growth rate of lepidopteran larvae depends on acquisition and utilization of ingested food (KAUSE *et al*. 1999) as well as on its conversion into larval biomass. This conversion, furthermore, depends on the activity of enzymes involved in protein, carbohydrate and lipid digestion.

The present study aimed to assess the adaptive significance of plasticity of total proteases, trypsin, leucine aminopeptidase, α-glucosidase and lipase of gypsy moth larvae (from *Quercus* and *Robinia* populations), in response to allelochemical stress imposed by feeding on a tannin-supplemented rearing diet. In order to quantify the intensity of selection on the specific activity and plasticity of these enzymes, we used relative growth rate from the third to fourth moulting, and relative growth rate during three days of the fifth instar, as fitness measures. We presumed that a higher relative growth rate is optimal and positively correlated with greater fitness of gypsy moth larvae.

MATERIALS AND METHODS

Experimental design

As previously described in MRDAKOVIĆ *et al*. (2013a, b), the experiment involved gypsy moth larvae originating from two populations with different host-use histories. A total of 32 egg masses from an oak forest (*Quercus* population) and 26 from a locust-tree forest (*Robinia* population) were collected. From each egg mass 7-9 larvae were randomly assigned to groups reared on the control diet (HWG diet, according to O`DELL *et al*. 1985) or the HWG diet supplemented with 5% tannic acid (Sigma Aldrich, lot number 32K1248). Thus, four experimental groups were created: *Quercus* larvae reared either on the control (QC) or the tannin-supplemented diet (QT), and *Robinia* larvae reared on the control (RC) and the tannin-supplemented diet (RT). Larvae were kept in Petri dishes (9cm diameter) at 23°C with a 12^h light : 12^h dark photoperiod. Fresh food was supplied daily.

The following traits were measured for each larva: relative growth rate from 3rd to 4th moult - RGR_1 and relative growth rate during 3 days of the 5th instar - RGR_2. RGR was calculated as: $RGR = (\ln M_t - \ln M_0) / t$, where M_0 and M_t were masses at the beginning and end of the examined period and t was time interval in days. For RGR_1, t represents the duration of the 4th instar, and for RGR_2 it is equal to 3 days. RGR_1 and RGR_2 were used as the measures of larval fitness.

On the third day of the fifth instar, larvae were sacrificed; their midguts were removed by dissection and homogenized individually in 0.15 mol NaCl (final tissue concentration – 100 mg/ml). The supernatants (crude midgut extracts) were used to determine the specific activity of digestive enzymes: total proteases (TP) (KUNITZ 1947), trypsin (TRY) and leucine aminopeptidase (LAP) (ERLANGER *et al*. 1961), alpha-glucosidase (α-G) (BAKER 1991) and lipase (LIP) (MRDAKOVIĆ *et al*. 2008). Specific enzyme activity, measured for each larval midgut extract in all experimental groups, was expressed as U/mg of midgut protein. Protein content was determined according to the method of BRADFORD (1976).

Statistical analyse

To quantify the intensity and direction of phenotypic selection acting on the enzyme activity of gypsy moth larvae, standardized selection gradients were estimated for each of the experimental environments (control and treatment), in both *Quercus* and *Robinia* populations (SAS 2003). As coefficients of partial regression, standardized selection gradients quantify (in units of phenotypic standard deviation; z - transformation, SOKAL and ROHLF 1981) the effect of each trait

on relative fitness, holding the effects of all other traits constant (LANDE and ARNOLD 1983). Regression models were used to test for directional selection.

Following SCHLICHTING (1986), plasticity was measured as the absolute value of the differences in family mean enzyme phenotype between the two treatments (control and tannin-supplemented diets). The simplified statistical model of SCHEINER and BERRIGAN (1998) was used to test for cost of plasticity of enzyme activities within each of the treatments and each of the populations: $W = Constant + \beta_1 \times X + \beta_2 \times X^2 + \beta_3 \times plX$, where W was the relative fitness in one environment/population, X was the value of the trait in the complementary environment/population, and plX was the between-treatment plasticity of the analyzed trait. Significant regression coefficients β_1 and β_2 measure direct selection on the trait value and account for the linear and non-linear components of selection, while β_3 describes how the ability to be plastic affects fitness. Since quadratic terms were non-significant in preliminary tests, they were removed from the analyses.

Relative fitness was estimated as the ratio of individual absolute fitness and average absolute fitness in both environments (C and T) within populations. The same procedure for estimating relative fitness of individuals was used in both phenotypic selection analysis and plasticity cost analysis. Differences of standardized regression coefficients between diets within populations (QC-QT, RC-RT), and between the two populations on each diet (QC-RC, QT-RT) were tested by the analysis of covariance (SAS 2003).

RESULTS

Selection gradients

Relative growth rates (RGR_1 and RGR_2) of gypsy moth larvae from both *Quercus* and *Robinia* populations decreased significantly in response to the tannin-supplemented rearing diet (Table 1) (MRDAKOVIĆ *et al.* 2013a, and herein). Selection analyses were performed in order to assess the adaptive significance of digestive enzyme plasticity, i.e. its contribution to higher larval fitness in the two environments. The phenotypic selection model applied to *Quercus* larvae in both control and stressful environments revealed that increased leucine aminopeptidase (LAP) activity was associated with higher RGR_1 and RGR_2 (Table 2a and 3a), i.e. higher relative fitness. The positive functional relationship between activity of lipase (LIP) and RGR_1, detected in *Quercus* larvae reared on the tannin-supplemented diet (Table 2a), suggests a positive fitness effect of higher LIP activity in the stressful environment. Significantly increased lipase activity and the trend of increasing leucine aminopeptidase activity, which we have already found in *Quercus* larvae reared on the tannin-supplemented diet (Table 1), (MRDAKOVIĆ *et al.* 2013a, b, and herein), may be considered as adaptive responses to allelochemical stress. In the *Robinia* population, decreased trypsin (TRY) activity was correlated with higher RGR_1 (Table 2a) and RGR_2 (Table 3a) while increased alpha-glucosidase (α-G) activity was associated with higher RGR_2 (Table 3a) in the control environment. Selection gradients for enzyme activities of gypsy moth larvae from the *Robinia* population failed to be detected as significant in the stressful environment (Table 2a and Table 3a), i.e. the plasticity of the enzyme activities was selectively neutral.

Low values of selection gradients observed in the phenotypic selection analysis resulted in predominantly non-significant differences in selection gradients between the experimental groups. Significant differences were found only between the two populations within the control environment for trypsin (TRY) and leucine aminopeptidase (LAP) (QC-RC comparison, Table

2B), and for alpha-glucosidase (α-G) (QC-RC comparison, Table 3B). The adaptive lipase (LIP) response to the tannin-supplemented rearing diet differed significantly between control and stressful conditions in the *Quercus* population (QC-QT comparison, Table 2B). A significant between-environment difference was detected for trypsin (TRY) activity in the *Robinia* population (RC-RT comparison, Table 2b).

Table 1. Means and standard errors (X ± SE) of relative growth rate (RGR$_1$ and RGR$_2$) and specific activity of digestive enzymes in gypsy moth larvae from Quercus and Robinia populations, reared on the control (C) and on the tannin-supplemented (T) diets. Values marked with different letters indicate significant differences among experimental groups within a row (P < 0.05, Sheffes`s multiple range test). Abbreviations are explained in Material and Methods.

		Quercus population					*Robinia* population			
		C		T			C		T	
	N	X ± SE	N	X ± SE		N	X ± SE	N	X ± SE	
[1]RGR$_1$	175	0.216	0.003[a]	207	0.187	0.002[b]	160	0.218	0.003[a]	180	0.190	0.002[b]
RGR$_2$	177	0.300	0.005[a]	221	0.263	0.003[b]	164	0.293	0.004[a]	185	0.263	0.003[b]
[1]TP	215	1.824	0.042[ab]	238	1.557	0.040[c]	176	1.996	0.055[a]	193	1.746	0.045[b]
[2]TRY	213	0.387	0.010[ab]	239	0.307	0.007[c]	180	0.418	0.013[a]	198	0.353	0.009[b]
[2]LAP	218	0.633	0.014[a]	244	0.671	0.015[a]	179	0.622	0.014[a]	189	0.656	0.014[a]
[1]α-G	217	1.058	0.030[a]	236	0.865	0.024[b]	174	0.905	0.032[b]	189	0.881	0.030[b]
[1]LIP	212	0.918	0.020[a]	223	1.133	0.029[b]	175	0.885	0.023[a]	195	1.289	0.027[c]

[1,2]Results for RGR$_1$ as well as for specific enzyme activities were published previously (MRDAKOVIĆ *et al.* 2013a, b).

Table 2. Standardized linear (β`) selection gradients for digestive enzyme specific activity in gypsy moth larvae from two populations, reared on the control (C) and on the tannin-supplemented (T) diets (within a row) (A.); F-values obtained from ANCOVA for comparison of β` between the diets within each population (QC-QT, RC-RT), and between two populations within each diet (QC-RC, QT-RT) (B.). Relative growth rate RGR$_1$ was the measure of fitness. Abbreviations are explained in Material and Methods.

	A.	*Quercus* population		*Robinia* population		**B.**			
		C	T	C	T	QC-QT	RC-RT	QC-RC	QT-RT
β`	TP	-0.020	-0.030	0.061	-0.002	0.06	2.32	3.48	0.47
	TRY	-0.024	-0.004	**-0.135**[***]	-0.003	0.21	**8.13**[**]	**5.52**[*]	0.00
	LAP	**0.073**[***]	**0.042**[*]	0.016	0.015	1.16	0.00	**4.11**[*]	0.79
	α-G	-0.019	0.002	0.010	0.004	0.68	0.05	1.18	0.01
	LIP	0.018	**0.071**[***]	0.034	0.030	**4.61**[*]	0.06	0.37	3.14

*P < 0.05, **P < 0.01, ***P < 0.001

*Table 3. Standardized linear (β`) selection gradients for digestive enzyme specific activity in gypsy moth larvae from two populations, reared on the control (C) and on the tannin-supplemented (T) diets (within a row) (**A.**); F-values obtained from ANCOVA for comparison of β` between the diets within each population (QC-QT, RC-RT), and between two populations within each diet (QC-RC, QT-RT) (**B.**). Relative growth rate RGR$_2$ was the measure of fitness. Abbreviations are explained in Material and Methods.*

	A.	*Quercus* population		*Robinia* population		**B**.			
		C	T	C	T	QC-QT	RC-RT	QC-RC	QT-RT
β`	TP	0.008	-0.039	0.010	-0.003	1.00	0.11	0.00	0.83
	TRY	-0.033	0.023	**-0.085***	-0.010	1.31	2.76	0.96	0.63
	LAP	**0.074****	**0.055****	0.028	0.027	0.38	0.00	2.16	0.94
	α-G	-0.030	-0.016	**0.044***	0.000	0.28	3.06	**6.48***	0.50
	LIP	0.039	0.021	0.013	-0.012	0.46	1.08	0.92	1.99

*P < 0.05, **P < 0.01

Cost of plasticity

In the stressful conditions, none of the regression coefficients were significant in the *Quercus* population (Table 4a). The active plastic responses of leucine aminopeptidase (LAP) (Table 2a and Table 3a) and lipase (LIP) (Table 2a) to stressful conditions did not incur any plasticity costs in the *Quercus* population. Moreover, the significant positive regression coefficient for lipase activity, detected in *Quercus* larvae in the control environment, points to the cost of homeostasis (Table 4a), although no significant selection gradient was revealed in phenotypic selection analyses (Table 2a). Similarly, in the *Robinia* population we found no evidence for a cost of plasticity of enzyme activities (Table 4a and Table 5a).

*Table 4. Regression analysis testing for plasticity costs of digestive enzyme specific activity in gypsy moth larvae from Quercus (Q) and Robinia (R) populations, reared on the control (C) and on the tannin-supplemented (T) diets (within a row) (**A.**); F-values obtained from ANCOVA for comparison of standardized regression coefficients for plasticity costs between the diets within each population (QC-QT, RC-RT), and between two populations within each diet (QC-RC, QT-RT) (**B.**). Relative growth rate RGR$_1$ was the measure of fitness. Abbreviations are explained in Material and Methods.*

	A. Regression coefficients				**B**. Comparison			
	QC	QT	RC	RT	QC-QT	RC-RT	QC-RC	QT-RT
TP	-0.020	-0.017	-0.017	0.005	0.01	1.03	0.01	1.48
TRY	0.006	-0.015	-0.032	0.002	1.04	2.49	2.35	0.92
LAP	0.009	0.004	-0.001	-0.014	0.07	0.40	0.20	1.02
α-G	0.031	-0.002	-0.025	-0.020	2.72	0.05	**5.62***	0.95
LIP	**0.033***	0.011	0.011	-0.017	1.09	1.40	0.82	1.73

*P < 0.05

*Table 5. Regression analysis testing for plasticity costs of digestive enzyme specific activity in gypsy moth larvae from Quercus (Q) and Robinia (R) populations, reared on the control (C) and on the tannin-supplemented (T) diets (within a row) (**A.**); F-values obtained from ANCOVA for comparison of standardized regression coefficients for plasticity costs between the diets within each population (QC-QT, RC-RT), and between two populations within each diet (QC-RC, QT-RT) (**B.**). Relative growth rate RGR$_2$ was the measure of fitness. Abbreviations are explained in Material and Methods.*

| | **A.** Regression coefficients | | | | **B**. Comparison | | | |
	QC	QT	RC	RT	QC-QT	RC-RT	QC-RC	QT-RT
TP	-0.006	-0.002	-0.031	-0.007	0.03	1.32	0.94	0.08
TRY	0.004	0.016	0.012	0.004	0.027	0.13	0.08	0.46
LAP	-0.007	0.015	-0.006	0.024	1.12	2.25	0.00	0.27
α-G	-0.018	-0.012	0.000	0.016	0.08	0.65	0.47	2.46
LIP	0.028	0.013	-0.008	0.002	0.40	0.21	1.96	0.28

DISCUSSION

As a generalist species, the gypsy moth encounters great variation in nutrient and allelochemical contents in host plant leaves. Oak species are highly preferred hosts, while the locust-tree is avoided by gypsy moth larvae. Locust leaves contain large quantities of tannins and have a lower nutrient value than oak leaves (MONTGOMERY 1986; UNRUH-SNYDER *et al*. 2007). Tannins affect feeding behavior and physiology of phytophagous insects through different mechanisms. They can act as phagodeterrents, digestibility reducers and oxidative stressors. Various mechanisms of defense in the larval midgut allow gypsy moths to overcome the harmful effects of tannin oxidation, so this species is considered to be a tannin-tolerant insect (BARBEHENN and CONSTABEL 2011). However, in the processes of development of environmentally safe and effective biological control agents for insect pests, the influence of tannins should be taken into account, as they significantly affect the sensitivity of gypsy moth larvae to bacterial and viral infections (BRODERICK *et al*., 2003; MARTEMYANOV *et al*. 2006).

Higher allocation of resources towards induction of defense mechanisms affects individual performance in stressful conditions (HOFFMANN and PARSONS 1991). Reduction of relative growth rate in gypsy moth larvae exposed to unsuitable host plants was suggested to be a consequence of high metabolic costs of the biochemical and physiological processes associated with induction of digestive and detoxification enzymes (LAZAREVIĆ *et al*. 2002; LAZAREVIĆ and PERIĆ-MATARUGA 2003). In this study, we analyzed the adaptive plastic response of digestive enzymes to allelochemical stress, related to beneficial higher growth rate of gypsy moth larvae. Much evidence suggested that lepidopteran larvae do not grow at the physiologically maximal rate, and their growth rate was found to be optimized by natural selection (FISCHER *et al*. 2004; GOTTHARD 2008).

Our analysis of phenotype selection indicates adaptive higher leucine aminopeptidase activity and production of more amino acids for uptake in *Quercus* larvae, as well as lower trypsin activity in *Robinia* larvae fed on artificial diet with a protein surplus. This enables allocation of more resources toward maximal weight gain, which is a characteristic for later larval instars. The higher α-glucosidase activity, connected with increased relative growth rate RGR$_2$, i.e. greater

relative fitness of larvae from the *Robinia* population, was detected in the control environment. Gypsy moths from the *Robinia* population had persisted in the locust-tree forest for more than 50 years (generations) (SIDOR and JODAL 1983), and adaptive enhanced α-glucosidase activity could be the consequence of stress selection, i.e. consumption by previous generations of leaves with high allelochemical but low protein and free sugar contents. Elevated enzyme activity should provide better utilization of dietary carbohydrate in such conditions. Significant between-population differences were recorded for plastic responses of these enzymes in the control treatment, suggesting long-term divergent evolution of the two populations under different conditions. Positive selection for higher leucine aminopeptidase activity of larvae from the *Quercus* population was also detected in the stressful environment. Similar to our results, adaptive induction of leucine aminopeptidase was found in *Helicoverpa armigera* larvae fed on various non-host plant (LOMATE and HIVRALE 2011) and diets containing plant proteinase inhibitors (HIVRALE *et al.* 2013). ZHU-SALZMAN *et al.* (2005) pointed out that plasticity of peptidases is a very important mechanism by which larvae phenotypically match nutritional needs in the presence of defense compounds in their food, which is in accordance with our results for aminopeptidase activity in *Quercus* larvae reared on the tannin-supplemented diet. Insects strongly regulate their intake of dietary macromolecules, while the effects of ingested allelochemicals depend on the protein : carbohydrate ratio (SIMPSON and RAUBENHEIMER 2001; BEHMER *et al.* 2002).

Dietary lipids are very important source of essential polyunsaturated fatty acids and the energy for larval growth (CHRISTELLER *et al.* 2011), particularly for later gypsy moth instars (STOCKHOFF 1993a). We recorded adaptive increase in lipase activity that enabled acquisition of sufficient energy resources from dietary lipids, necessary for induction of defense mechanisms and faster growth of *Quercus* larvae from the third to fourth moult in the stressful environment. Surprisingly, such an adaptive response was not connected with higher relative growth rate during three days of the fifth instar. Enhanced midgut lipase activity was reported for *Glyphodes pyloalis* larvae treated with various concentrations of *Artemisia annua* extract (KHOSRAVI *et al.* 2011), and in response to plant essential oils, providing greater utilization of dietary lipids in biomass production (YAZDANI *et al.* 2013).

This study did not find any evidence for plasticity costs, while the cost of homeostasis recorded for lipase activity in *Quercu*s larvae suggested that an active plastic response in lipase activity would confer higher larval fitness in the control environment. The selectively favored sensitivity of leucine aminopeptidase and lipase activity levels, revealed in *Quercus* larvae under allelochemical stress, indicated great potential for evolution of digestive enzyme plasticity in gypsy moth larvae. Even when it evolves in suitable habitats (oak forests) this extremely generalist species with outbreaking population dynamics is exposed to periodical exhaustion of suitable hosts. Significant plasticity of physiological traits is important characteristic of insect generalists (KARBAN and AGRAWAL 2002), and the adaptive plasticity of digestive enzyme activity in gypsy moth larvae recorded in the present work might enable optimal larval growth in the heterogeneous and stressful environments they frequently encounter.

REFERENCES

BAKER, J.E. (1991): Properties of glycosidases from the maize weevil, *Sitophilus zeamais*. Insect Biochem, *21*:615-621.

BARBEHENN, R.V., P.C. CONSTABEL (2011): Tannins in plant-herbivore interactions. Phytochemistry, *72*:1551–1565.

BARBOSA, P., V.A. KRISCHIK (1987): Influence of alkaloids on feeding preference of eastern deciduous forest tree by the gypsy moth *Lymantria dispar* L. Am Nat, *130*:53-59.

BEHMER, S.T., S.J. SIMPSON, D. RAUBENHEIMER (2002): Herbivore foraging in chemically heterogeneous environments: nutrients and secondary metabolites. Ecology, *83*:2489-2501.

BERBEHENN,R.V., J. KOCHMANSKI (2013): Searching for synergism: effects of combinations of phenolic compounds and other toxins on antioxidative stress in *Lymantria dispar* caterpillars. Chemoecology, *23*:219-231.

BOURCHIER, R.S., V.G. NEALIS (1993): Development and growth of early- and late-instar of gypsy moth (Lepidoptera: Lymantriidae) feeding on tannin-supplemented diets. Environ Entomol, *22*:642-646.

BRADFORD, M. (1976): A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, *72*:248-254.

BRODERICK, N.A., R.M. GOODMAN, J. HANDELSMAN, K.F. RAFFA (2003): Effect of Host Diet and Insect Source on Synergy of Gypsy Moth (Lepidoptera: Lymantriidae) Mortality to *Bacillus thuringiensis* subsp. *kurstaki* by Zwittermicin A. Environ Entomol, *32*:387-391.

CHRISTELLER, J.T., S. AMARA, F. CARRIERE (2011): Galactolipase, phospholipase and tryacylglycerol lipase activities in the midgut of six species of lepidopteran larval feeding on different lipid diets. J Insect Physiol, *57*:1232-1239.

ERLANGER, B.F., N. KOKOWSKI, W. COHEN (1961): The preparation and properties of two new chromogenic substrates of trypsin. Arch Biochem Biophys, *95*:271-278.

FISCHER, K., I. ZEILSTRA, S.K. HETZ, K. FIEDLER (2004): Physiological costs of growing fast: does accelerated growth reduce pay-off in adult fitness? Evol Ecol, *18*:343-353.

GOTTHARD, K. (2008): Adaptive Growth Decisions in Butterflies. BioScience, *58*:222-230.

HEMMING, J.D.C., R.L. LINDROTH (1995): Intraspecific Variation in Aspen Phytochemistry: Effects on Performance of Gypsy Moths and Forest Tents Caterpillars. Oecologia, *103*:79-88.

HEMMING, J.D.C., R.L. LINDROTH (2000): Effects of Phenolic Glycosides and Protein on Gypsy Moth (Lepidoptera: Lymantriidae) and Forest Tent Caterpillar (Lepidoptera: Lasiocampidae) Performance and Detoxication Activities. Environ Entomol, *29:*1108-1115.

HIVRALE, V.K., P.R. LOMATE, S.S. BASAIYYE, N.D. KALVE (2013): Compensatory proteolytic responses to dietary proteinase inhibitors from *Albizia lebbeck* seeds in the *Helicoverpa armigera* larvae. Arthropod-Plant Inte, *7*:259-266.

HOFFMANN, A.A., P.A. PARSONS (1991): Evolutionary genetics and environmental stress. Oxford University Press, Oxford.

JANKOVIĆ-TOMANIĆ, M, J. LAZAREVIĆ (2012): Effects of Temperature and Dietary Nitrogen on Genetic Variation and Covariation in Gypsy Moth Larval Performance Traits. Arch Biol Sci, *64*:1109-1116.

KARBAN, R, A.A. AGRAWAL (2002): Herbivore Offense. Annu Rev Ecol Syst, *33*:641-664.

KAUSE, A, I. SALONIEMI, E. HAUKIOJA, S. HANHIMÄKI (1999): How to become large quickly: quantitative genetics of growth and foraging in a flush feeding lepidopteran larva. J Evol Biol, *12*:471-482.

KHOSRAVI, R., J.J. SENDI, M. GHADAMYARI, E. YEZDANI (2011): Effect of Sweet Wormwood *Artemisia annua* Crude Leaf Extracts on Some Biological and Physiological Characteristics of the Lesser Mulberry Pyralid, *Glyphodes pyloalis*. J Insect Sci, *11*:1-13.

KINGSOLVER, J.G., R. IZEM, G.J. RAGLAND (2004): Plasticity of Size and Growth in Fluctuating Thermal Environments: Comparing Reaction Norms and Performance Curves. Integr Comp Biol, 44:450-460.

KUNITZ, M. (1947): Crystalline soybean trypsin inhibitor. II. General properties. J Gen Physiol, 30:291-310.

LANDE, R., S.J. ARNOLD (1983): The measurement of selection on correlated characters. Evolution, 37:1210-1226.

LAZAREVIĆ, J. (2000): Physiological and Genetic Mechanisms of Adaptation to Unsuitable Nutrition in the Gypsy Moth, *Lymantria dispar* L. PhD, (Abstract in English), Faculty of Biology, University of Belgrade, Belgrade, Serbia.

LAZAREVIĆ, J., V. PERIĆ-MATARUGA (2003): Nutritive stress effects on growth and digestive physiology of *Lymantria dispar* larvae. Jugoslav Med Biochem, *22*:53-59.

LAZAREVIĆ, J., V. PERIĆ-MATARUGA, B. STOJKOVIĆ, N. TUCIĆ (2002): Adaptation of the gypsy moth to an unsuitable host plant. Entomol Exp Appl, *102*:75-86.

LAZAREVIĆ, J., V. PERIĆ-MATARUGA, Z. PROLIĆ, N. TUCIĆ (2003): Behavioural response to an unsuitable host plant in the gypsy moth (*Lymantria dispar* L.). Folia Biol-Krakow, *51*:129-131.

LOMATE P.R., V.K. HIVRALE (2011): Differential responses of midgut soluble aminopeptidases of *Helicoverpa armigera* to feeding on various host and non-host plant diets. Arthropod-Plant Inte, *5*:359-368.

MARGRAF, N., K. GOTTHARD, M. RAHIER (2003): The growth strategy of an alpine beetle: maximization or individual growth adjustment in relation to seasonal time horizons? Funct Ecol, *17*:605-610.

MARTEMYANOV, V.V., S.A. BAKHVALOV, I.M. DUBOVSKIY, V.V. GLUPOV, N.F. SALAKHTUDINOV, G.A. TOLSTIKOV (2006): Effect of Tannic Acid on the Development and Resistance of the Gypsy Moth *Lymantria dispar* L. to Viral Infection. Dokl Biochem Biophys, *409*:219-222.

MONTGOMERY, M.E. (1986): Gypsy moth host plant relationships and population dynamics. *In*: Proceeding 18th IUFRO World Congress, Division 2, Vol. II, Forest Plants and Forest Protection. Ljubljana, Yugoslavia, 743-754.

MRDAKOVIĆ, M. (2010): The Evolution of Phenotypic Plasticity in Response to Nutritive Stress in the Gypsy Moth *Lymantria dispar* L. Larvae. PhD, (Abstract in English), Faculty of biology, University of Belgrade, Belgrade, Serbia.

MRDAKOVIĆ, M., B. STOJKOVIĆ, V. PERIĆ-MATARUGA, L. ILIJIN, M. VLAHOVIĆ, J. LAZAREVIĆ (2014): Adaptive phenotypic plasticity of gypsy moth digestive enzymes. Cent Eur J Biol, *9*:309-319.

MRDAKOVIĆ, M., J. LAZAREVIĆ, V. PERIĆ-MATARUGA, L. ILIJIN, M. VLAHOVIĆ (2008): Partial characterization of a lipase from gypsy moth (*Lymantria dispar* L.) larval midgut. Folia Biol-Krakow, *56*:103-110.

MRDAKOVIĆ, M., V. PERIĆ-MATARUGA, L. ILIJIN, M. VLAHOVIĆ, D. TODOROVIĆ, V. NENADOVIĆ, J. LAZAREVIĆ (2011): The effects of tannic acid on the fitness-related traits of *Lymantria dispar* L. larvae. Arch Biol Sci, *63*:1037-1045.

MRDAKOVIĆ M., V. PERIĆ-MATARUGA, L. ILIJIN, M. VLAHOVIĆ, D. TODOROVIĆ, V. NENADOVIĆ, J. LAZAREVIĆ (2013b): Effects of tannic acid on trypsin and leucine aminopeptidase activities in gypsy moth larval midgut. Arch Biol Sci, *65*:1405-1413.

MRDAKOVIĆ M., V. PERIĆ-MATARUGA, L. ILIJIN, M. VLAHOVIĆ, M. JANKOVIĆ-TOMANIĆ, D. MIRČIĆ, J. LAZAREVIĆ (2013a): Response of *Lymantria dispar* (Lepidoptera: Lymantriidae) larvae from differently adapted populations to allelochemical stress: Effects of tannic acid. Eur J Entomol, *110*:55-63.

NIJHOUT, H.F. (1994): Insect hormones. Princeton University Press, Princeton, New Jersey.

NYLIN, S., K. GOTTHARD (1998): Plasticity in life-history traits. Annu Rev Entomol, *43*:63-83.

O`DELL, T.M., C.A. BUTT, A.W. BRIDGEFORTH (1985): *Lymantria dispar*. *In*: P. Singht, R. Moore (eds): Handbook of Insect Rearing. Elsevier, New York, USA, 355-367.

OJEDA-AVILA, T., A.H. WOODS, R.A. RAGUSO (2003): Effects of dietary variation on growth, composition, and maturation of *Manduca sexta* (Sphingidae: Lepidoptera). J Insect Physiol, *49*:293-306.

SAS Institute Inc. (2003): The SAS System for Windows. Release 9.1. SAS Institute, Cary, NC, USA.

SCHEINER, S.M., D. BERRIGAN (1998): The genetics of phenotypic plasticity. VIII. The cost of plasticity in *Daphnia pulex*. Evolution, *52*:545-555.

SCHEIRS, J., L. DE BRUYN, R.VERHAGEN (2002): Seasonal changes in leaf nutritional quality influence grass miner performance. Ecol Entomol, *27*:84-93.

SCHLICHITING, C.D. (1986): The evolution of phenotypic plasticity in plants. Annu Rev Ecol Syst, *17*:667-694.

SIDOR, Ć, I. JODAL (1983): Results of investigations of health conditions of gypsy moth (*Porthetria dispar* L.) in accacia forest "Bagremara". Plant Protect, *34:*445-455.

SIMPSON, S.J., D. RAUBENHEIMER (2001): The geometric analysis of nutrient-allelochemical interactions: a case study using locusts. Ecology, *82*:422-439.

SOKAL, R.R., F.J. ROHLF (1981): Biometry. Freeman, San Francisco.

STEARNS, S.C. (1992): The Evolution of Life Histories. Oxford University Press, Oxford.

STOCKHOFF, B. (1992): Diet-switching by gypsy moth: effects of diet nitrogen history vs. switching on growth, consumption, and food utilization. Entomol Exp Appl, *64*:225-238.

STOCKHOFF, B. (1993a): Ontogenetic Change in Dietary Selection for Protein and Lipid by Gypsy Moth Larvae. J Insect Physiol, *39*:677-686.

STOCKHOFF, B. (1993b): Diet heterogeneity: implications for growth of a generalist herbivore, the gypsy moth. Ecology, *74*:1939-1949.

TAMMARU, T., K. RUOHOMÄKI, M. MONTOLA (2000): Crowding-Induced Plasticity in *Epirrita autumnata* (Lepidoptera: Geometridae): Weak Evidence of Specific Modifications in Reaction Norms. Oikos, *90*:171-181.

TAMMARU, T., S. NYLIN, K. RUOHOMÄKI, K. GOTTHARD (2004): Compensatory responses in lepidopteran larvae: a test of growth rate maximization. Oikos, *107:*352-362.

UNRUH-SNYDER, L.J., L-M. LUGINBUHL, J.P. MUELLER, A.P. CONRAD, K.E. TURNER (2007): Intake, digestibility and nitrogen utilization of *Robinia pseudoacacia* foliage fed to growing goat wethers. Small Ruminant Res, *71*:179-193.

VANHANEN, H., T.O. VETELI, S. PÄIVINEN, S. KELLOMÄKI, P. NIEMELÄ (2007): Climate change and range shifts in two insect defoliators: gypsy moth and nun moth – a model study. Silva Fenn, *41*:621-638.

YAZDANI E., J.J. SENDI, A. ALIAKBAR (2013): Chemical composition, toxicity and physiological effects of essential oil of *Rosemarinus officinalis* on lesser mulberry pyralid, *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae). J Crop Protect, *2*:461-467.

ZHU-SALZMAN K., J-L. BI, T-X. LIU (2005): Molecular strategies of plant defense and insect counter-defense. Insect Sci, *12*:3–15.

TESTIRANJE ADAPTIVNE PLASTIČNOSTI DIGESTIVNIH ENZIMA GUBARA U ODGOVORU NA TANINSKU KISELINU UZ POMOĆ FENOTIPSKE SELEKCIONE ANALIZE

Marija MRDAKOVIĆ[1*], Biljana STOJKOVIĆ[2,3], Larisa ILIJIN[1], Milena VLAHOVIĆ[1], Vesna PERIĆ-MATARUGA[1], Jelica LAZAREVIĆ[1]

[1]Odeljenje za fiziologiju i biohemiju insekata, Institut za biološka ustraživanja "Siniša Stanković", Univerzitet u Beogradu, Beograd, Srbija
[2]Odeljenje za evolucionu biologiju, Institut za biološka istraživanja "Siniša Stanković", Univerzitet u Beogradu, Beograd, Srbija
[3]Biološki fakultet, Univerzitet u Beogradu, Beograd, Srbija

Izvod

Adaptivni značaj plastičnosti aktivnosti digestivnih enzima u odgovoru na alelohemikalijski stres testiran je na 32 ful-sib familije gubara iz hrastove šume (*Quercus* populacija) i 26 familija iz bagremove šume (*Robinia* populacija), gajenih na kontrolnoj dijeti i dijeti sa taninskom kiselinom. Fenotipska selekciona analiza u kojoj je relativna brzina rasta predstavljala meru adaptivne vrednosti, pokazala je adaptivnost veće specifične aktivnosti leucin aminopeptidaze kod *Quercus* larvi i manje specifične aktivnosti tripsina kod *Robinia* larvi u kontrolnoj sredini, dok je adaptivni odgovor na prisustvo stresora podrazumevao povećanje aktivnosti leucin aminopeptidaze i lipaze kod *Quercus* larvi. Nije detektovana cena plastičnosti aktivnosti digestivnih enzima ni u jednoj eksperimentalnoj grupi. Dobijeni rezultati ukazuju da adaptivna plastičnost aktivnosti digestivnih enzima larvi gubara doprinosi optimalnoj brzini rasta u različitim uslovima sredine.