

## THE EFFECTS OF TANNIC ACID ON THE FITNESS-RELATED TRAITS OF *LYMANTRIA DISPAR* L. LARVAE

MARIJA MRDAKOVIĆ\*, VESNA PERIĆ MATARUGA, LARISA ILIJIN, MILENA VLAHOVIĆ, DAJANA TODOROVIĆ, VERA NENADOVIĆ and JELICA LAZAREVIĆ

*Department of Insect Physiology and Biochemistry, Institute for Biological Research "Siniša Stanković", University of Belgrade, 11060 Belgrade, Serbia*

**Abstract** - In this study we investigated the effects of tannic acid on fitness-related traits in gypsy moth larvae from two differently adapted populations. Thirty two full-sib families from oak (suitable host plant, *Quercus* population), and twenty six full-sib families from locust-tree (unsuitable host plant, *Robinia* population) forests were reared on artificial diets with or without a 5% tannic acid supplement. Tannic acid shortened the duration of larval development till the 4<sup>th</sup> instar in the *Robinia* population, and decreased the mass of larvae from both *Quercus* and *Robinia* populations. Local adaptation was not recorded for any of the examined traits. In general, regardless of population origin, genetic variation (broad-sense heritability) was higher in larvae fed on the diet containing tannic acid than in larvae fed on the control diet. Variability of phenotypic plasticity was significant for the examined traits, except for larval duration traits in *Quercus* larvae, pointing to the potential of gypsy moth larvae from both populations for the evolution of adaptive plastic responses to new environmental conditions and the presence of stressors. Genetic correlations between the environments were positive and significantly different from "one" and accordingly, do not represent constraint for the evolution of plasticity.

**Key words:** Gypsy moth, tannic acid, genetic variation, phenotypic plasticity

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### INTRODUCTION

Plant-insect interaction is a dynamic system subjected to continual variation and change. As a result of plant-insect coevolution, plants synthesize different allelochemicals to reduce insect attack, while insects use various strategies to overcome plant defense barriers, allowing them successful development and reproduction. This "arms-race" is the main factor in the diversification of both phytophagous insects and host plants (Ehrlich and Raven, 1964). Phenotypic plasticity has an important role in promoting evolutionary diversification (Crispo, 2008), and in phytophagous insects it can be expressed in response to variation of the nutritive and antinutritive traits of their host plants (Haribal and Renwick,

2005). Phytophagous insects can use many different host plants across their geographic distribution, but within their environments they usually use a small number of plant species (Ruiz-Montoya et al., 2003). Variation in the use of different host plants shows the patterns of local adaptation of insect populations and also indicates responses to past selection (Ueno et al., 2001).

Tannins are one of the most important classes of secondary plant compounds involved in the defense of deciduous plants against phytophagous insects. Exactly how tannins affect the herbivore is still open to debate, but they are referred to as antidigestive protein binding agents (Cardinal-Aucoin et al., 2009). The adverse effects of tannins have been shown for

different insect species, and during the course of evolution insects have acquired various defense mechanisms against their harmful effects (Barbehenn and Constabel, 2011, and references herein). The gypsy moth feeds on many tannin-containing tree leaves (Barbosa and Krischik, 1987), and numerous studies have been devoted to the influence of tannins on its performance (Rossiter et al., 1988; Bouchier and Nealis, 1993; Foss and Rieske, 2003; Barbehenn et al., 2009).

In the present study we investigated the effects of tannic acid on gypsy moth larvae from two populations with different trophic adaptation, originating from oak (suitable host plant) and locust-tree (unsuitable host plant) forests. The unfavorable effects of locust leaves on gypsy moth larvae can be attributed to the high level of allelochemicals (alkaloids, flavonoids, tannins) and low nutrient content (Barbosa and Krischik, 1987). Our goals were to examine the effects of tannic acid (hydrolysable tannins), a digestibility reducer and pro-oxidant compound, on fitness-related traits of the gypsy moths larvae from two populations, as well as differences in response to the nutritive stress related to different population origin. We examined the influence of nutritive stress on the genetic variability of the traits and differences in expression of this variability due to adaptations of the larvae from locust-tree population to the stress, variability of the phenotypic plasticity in gypsy moth larvae from the different populations that determines the potential for further evolution of trait plasticity, and we also assessed possible constraints on the evolution of trait plasticity.

## MATERIALS AND METHODS

### *Insect rearing*

Thirty two egg masses of gypsy moth were collected from a mixed oak forest (*Quercetum farnetto-cerris* Rudski 1949) (Stajić et al., 2008) within the Bogovadja forest complex, hereinafter referred to as the *Quercus* population, (locality Bogovadja, 70 km south-west of Belgrade), and twenty six egg masses collected from a man-made locust-tree (*Robinia pseudoacacia* L.)

forest, hereinafter referred to as the *Robinia* population, (locality "Bagremara" near Bačka Palanka, 122 km north-west of Belgrade). During the winter period, egg masses were kept at 4°C until May, when they were transferred to a constant temperature of 23°C to hatch. All larvae hatched from a single egg mass represent full-sibs. They were reared at a constant temperature of 23°C and 12L:12D photoperiod. Within each egg mass from both the *Quercus* and *Robinia* populations, 7-9 larvae (unbalanced full-sib design) were randomly assigned to the group fed on the control artificial diet (high wheat germ HWG diet, O'Dell et al., 1985) and the group fed on tannin supplemented HWG diet which contained 5% of tannic acid. Accordingly, four experimental groups were created: QC – *Quercus* larvae fed on the control diet, QT – *Quercus* larvae fed on the tannin supplemented diet, RC – *Robinia* larvae fed on the control diet, RT – *Robinia* larvae fed on the tannin supplemented diet.

### *Measurement of life history traits*

In order to estimate the effects of tannic acid on larvae from differently adapted populations, i.e. their responses at the level of fitness-related traits, the following traits were measured for each larva: duration of larval development (in days) from hatching till molting into the fourth instar-D4; duration of larval development (in days) from hatching till molting into the fifth instar-D5; larval mass at the beginning of the 4<sup>th</sup> instar- m4 and, larval mass at the beginning of the 5<sup>th</sup> instar- m5.

### *Statistical analysis*

Mean values and standard errors were determined for the analyzed traits. The experimental groups were compared using one-way ANOVA and Sheffé's multiple range test (Sokal and Rohlf, 1981). The acceptable P cutoff value was set at 0.05. Analysis of variance was applied to log transformed values of the traits. Local differentiation was tested by two-way ANOVA; population origin (Pop) and tannin treatment (T) were used as fixed factors. Broad-sense heritability was calculated according to the

**Table 1.** Means (X) and standard errors (SE) of fitness-related traits of gypsy moth larvae from two populations, reared on the control (C), and on the diet with 5% tannic acid (T). Abbreviations are explained in Materials and Methods.

Trait	<i>Quercus</i> population						<i>Robinia</i> population					
	C			T			C			T		
	N	X ± SE		N	X ± SE		N	X ± SE		N	X ± SE	
D 4	220	16.127	0.115 <sup>abc</sup>	241	15.983	0.126 <sup>ac</sup>	178	16.635	0.177 <sup>b</sup>	196	16.015	0.162 <sup>c</sup>
D 5	219	21.438	0.138 <sup>a</sup>	235	21.434	0.148 <sup>a</sup>	177	22.006	0.192 <sup>a</sup>	194	21.412	0.187 <sup>a</sup>
m 4	201	103.398	2.001 <sup>a</sup>	219	99.251	1.359 <sup>ab</sup>	172	100.076	1.722 <sup>ab</sup>	188	96.590	1.497 <sup>b</sup>
m 5	203	324.355	8.423 <sup>a</sup>	234	273.197	4.393 <sup>b</sup>	171	325.251	7.459 <sup>a</sup>	194	267.536	5.161 <sup>b</sup>

a, b, c – values marked with different letters differ significantly (Scheffe's multiple range test).

standard formulae for unbalanced full-sibling design (Becker, 1984). Comparison of trait heritabilities between the diets within each of the populations (QC-QT; RC-RT), and between two populations on each of the diets (QC-RC; QT-RT), were performed on z-transformed values of heritabilities. Using the “family (Fam) × treatment (T)” interaction from two-way ANOVA, we evaluated whether individuals from different genotypes responded differently to the nutritive stress. Treatment was fixed and families were random factors. Differences in plastic responses among families (egg masses) were also represented by norm of reaction plots. The lines of norm of reaction showed full-sib responses of the traits to the nutritive stress in 32 families from the *Quercus* and 26 families from the *Robinia* population. The estimates of genetic correlation were calculated across different environments (control and diet with 5% tannic acid) with Pearson's product-moment method, using the families' means of the traits (Sokal and Rohlf, 1981). All estimates used came from the full-sib design and contained contributions from nonadditive genetic variance, maternal and environment effects.

## RESULTS

The obtained results show that tannic acid in the diet shortened the duration of development from molt-

ing till hatching into 4<sup>th</sup> instar *Robinia* larvae ( $F_{3, 831} = 4.059$ ,  $P < 0.001$ ), while the D4 of the *Quercus* larvae did not change significantly in response to tannic acid. Larvae from both the *Quercus* and *Robinia* populations had a significantly lower larval mass at the beginning of the 5<sup>th</sup> instar ( $F_{3, 798} = 23.800$ ,  $P < 0.0001$ ) when they were reared on the diet with 5% tannic acid, compared to those reared on the control diet (Table 1).

Two-way ANOVA, with population and tannin treatment as the factors, did not reveal significant population or “population × treatment” effects for any of the analyzed traits (Table 2). The absence of a significant “Pop×T” interaction showed that the populations reacted in a similar way to the nutritive stress. Significant treatment effects were shown for D4 and m5.

The broad-sense heritabilities, estimated for larval development duration traits and masses, are presented in Table 3. The majority of heritabilities were moderate or high, ranging from 0.300 to 0.800. The exception was  $h^2$  for m5 of *Robinia* larvae reared on the control diet. Heritability increased and became significant for m5 of *Robinia* larvae in the presence of tannic acid in the diet. In both populations the heritability of most of the estimated traits showed higher

**Table 2.** Mean squares ( $\times 10^3$ ) from two way ANOVA of fitness-related traits of gypsy moth larvae from two populations reared on the control, and on the diet with 5% tannic acid; population origin (Pop) and treatment (T) are fixed factors.

Trait		Source of variation			
		Pop	T	Pop $\times$ T	Error
D 4	d.f.	1	1	1	831
	MS	6.836	23.077	7.126	2.878
	F	2.375	8.017**	2.476	
D 5	d.f.	1	1	1	821
	MS	4.427	8.107	6.806	2.190
	F	2.021	3.701	3.107	
m 4	d.f.	1	1	1	776
	MS	0.941	1.262	0.043	0.448
	F	2.102	2.820	0.096	
m 5	d.f.	1	1	1	798
	MS	0.002	34.659	0.820	0.460
	F	0.005	75.370***	1.783	

\*\*P<0.01, \*\*\* P<0.001

values in larvae reared on the diet with 5% tannic acid. Regardless of the diet, the heritability of larval masses was significantly higher in the *Quercus* than in the *Robinia* larvae (Table 3).

Two-way ANOVA, with family and treatment as the factors (Table 4), revealed a significant family effect for all analyzed traits, a significant treatment effect for m5, and significant "Fam $\times$ T" interactions for larval masses in the *Quercus* population. A sig-

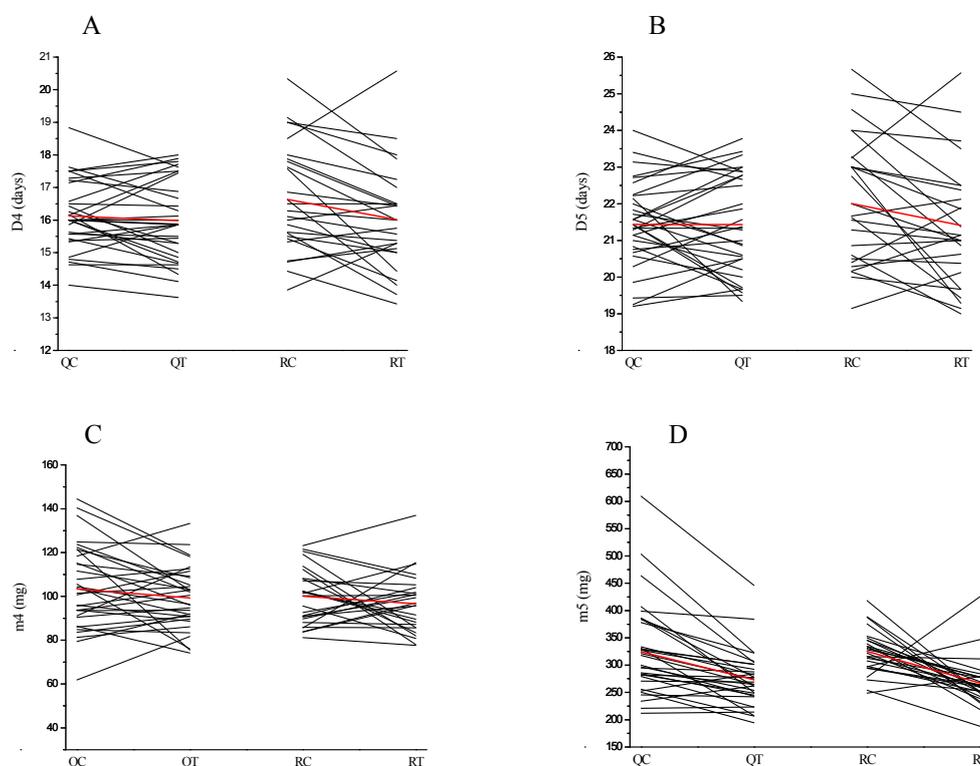
nificant family effect was also found for the analyzed traits in the *Robinia* population. Only m4 of *Robinia* larvae did not show a significant treatment effect, while significant "Fam $\times$ T" interactions were found for the analyzed traits of larvae from this population. Gypsy moth families from both populations displayed variation in cross-reaction norms for the larval fitness-related traits (Fig. 1A-D). In 19 families (out of 26) from the *Robinia* population the mean for D4 decreased in response to dietary tannic acid

**Table 3.** Broad sense heritabilities ( $\pm$ SE) of fitness-related traits of gypsy moth larvae from two populations reared on the control (C), and on the diet with 5% tannic acid (T).

Trait	<i>Quercus</i> population				<i>Robinia</i> population			
	C		T		C		T	
	$h^2$	SE	$h^2$	SE	$h^2$	SE	$h^2$	SE
D 4	0.530	0.152**	0.639	0.154***	0.759	0.180***	0.787	0.177***
D 5	0.358	0.136*	0.506	0.147**	0.684	0.179***	0.605	0.170***
m 4	0.732	0.166***	0.773	0.162***	0.374	0.157 <sup>A</sup>	0.637	0.173***
m 5	0.801	0.166***	0.792	0.160***	0.047	0.105 <sup>C</sup>	0.421	0.154 <sup>A</sup>

\* $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

A ( $P < 0.05$ ), C ( $P < 0.001$ ) - values marked with A and C depict significant differences of the trait  $h^2$  between *Robinia* and *Quercus* larvae within the same diet

**Figure 1.** Norm of reaction plots for the duration of larval development (A, B) and larval masses (C, D); each line depicts the average response of gypsy moth full-sib families from the *Quercus* and *Robinia* populations to the presence of tannic acid in the diet. Abbreviations are explained in Materials and Methods.

**Table 4.** Mean squares ( $\times 10^3$ ) from two way ANOVA of fitness-related traits of gypsy moth larvae from two populations reared on the control, and on the diet with 5% tannic acid; treatment (T) was fixed, and family (Fam) was random factor.

Trait	<i>Quercus</i> population				<i>Robinia</i> population				
	Fam	T	Fam×T	Error	Fam	T	Fam ×T	Error	
	d.f.	31	1	31	397	25	1	25	322
D 4	MS	11.524	3.158	1.807	1.667	20.760	32.303	3.722	2.193
	F	6.915***	1.747	1.084		9.467***	8.678**	1.698*	
	d.f.	31	1	31	390	25	1	25	319
D 5	MS	7.127	0.176	1.724	1.480	12.485	19.862	3.011	1.760
	F	4.814***	0.102	1.164		7.096***	6.595*	1.712*	
	d.f.	31	1	31	356	25	1	25	308
m 4	MS	2.447	0.954	0.630	0.764	1.252	0.673	0.736	0.750
	F	7.981***	1.513	2.056**		4.129***	0.914	2.427***	
	d.f.	31	1	31	373	25	1	25	313
m 5	MS	2.885	15.107	0.469	1.000	0.615	19.889	0.798	1.235
	F	9.662***	32.199***	1.571*		1.666*	24.923***	2.162**	

\* P&lt;0.05, \*\* P&lt;0.01, \*\*\* P&lt;0.001

**Table 5.** Genetic correlations across different environments (control, and diet with 5% tannic acid) for fitness-related traits of gypsy moth larvae from two populations.

Trait	<i>Quercus</i> population	<i>Robinia</i> population
D 4	0.746***	0.722***
D 5	0.636*** A	0.641***
m 4	0.581*** A	0.241
m 5	0.722***	-0.245

\*\*\*P&lt;0.001

Significant positive correlation coefficient differ from “one“ marked with letter A (P&lt;0.05).

(Fig. 1A), but both *Quercus* and *Robinia* populations showed similar family responses regarding m5. Thus, in 28 out of 32 families from the *Quercus* and 23 out of 26 families from the *Robinia* population, the means for m5 decreased in response to the nutritive stress (Fig. 1D).

Coefficients of genetic correlation across environments (control and diet with 5% tannic acid) within each population are presented in Table 4. Larval development duration traits D4 and D5 showed statistically significant positive correlations across environments in both *Quercus* and *Robinia* populations. In the *Quercus* population, larval masses m4 and m5 showed significant positive genetic correlations across environments. Significant genetic correlations across different environments, significantly less than “one”, were shown by D5 and m4 in the *Quercus* population. A negative, but statistically insignificant correlation across environments was recorded only for larval mass m5 in the *Robinia* population.

## DISCUSSION

The obtained results confirmed significant effects of nutritive stress (tannic acid - digestibility reducer and pro-oxidant compound) on gypsy moth fitness-related traits. Rearing gypsy moth larvae from the *Robinia* population on a diet supplemented with 5% tannic acid shortened the time of exposure of young and more vulnerable larvae to a negative effect of nutritive stress. Possibly, long-term feeding on tannin-rich locust-tree leaves imposed higher selective pressure on young sensitive larvae whose adaptive plasticity could be maintained through the Baldwin effect. As an optimal host plant for gypsy moth development, the oak tree does not require high plasticity of this trait. As neurohormones are an inevitable component of adaptation processes, selection may act on the hormonal level. Exogenous and endogenous factors that change the activity of neurosecretory neurons also change the hormonal balance and thereby the dynamic of insect development (Ivanović and Janković-Hladni, 1991). The effect of tannic acid was also expressed as a reduction of larval mass for both *Quercus* and *Robinia* popu-

lations, which might be the consequence of changes in digestibility, considering that the efficiency of food conversion into biomass depends on digestive enzyme activities. Significantly reduced activities of some digestive enzymes in gypsy moth larvae from both *Quercus* and *Robinia* populations were recorded in response to tannic acid in the diet (Mrdaković, 2010). It is considered that one mode of tannin action is pro-oxidant activity, and that tannin oxidation in the insect gut produces reactive oxygen species that can damage nutrients and/or midgut tissues, influencing insect performance (Barbehenn and Constabel, 2011). We assumed that the reduction of gypsy moth larval masses was due to tannic acid pro-oxidant activity and consequently the allocation of resources towards defense mechanisms and induction of components of the antioxidative system. Since the *Robinia* population of gypsy moth larvae has experienced locust-tree leaves as an exclusive food resource for more than 50 years (Sidor and Jodal, 1983), one might expect evidence of host plant specialization at the population level. Lazarević et al., (2002) found significant differences between the *Quercus* and *Robinia* populations in pupal duration and nutritional indices, and also a significant “population × host” interaction in preadult viability and duration of pupal stage, which suggest that the adaptation of the gypsy moth to the unsuitable host (locust-tree) might be ongoing. In addition, Perić-Mataruga et al. (1997) recorded population differences at the level of antioxidative enzyme activities and plasticity of the response to ingested allelochemicals that point to population-level specialization to locust-tree leaves. However, we did not detect significant population or “population × treatment” interaction effects for the examined fitness-related traits i.e., local adaptation with respect to tannic acid.

The amount of genetic variation in fitness components expressed in novel environments is important for adaptation to those environments and for broadening the ecological niche (Kawecki, 1995). Several hypotheses predict increased genetic variability in novel/stressful environments (Hoffmann and Merilä, 1999). We recorded significant moderate to high heritability for the majority of the examined fitness-

related traits of gypsy moth larvae from both populations and most of them tended to increase in larvae reared on the diet with 5% tannic acid, although without significant differences between the diets within the populations. Also, comparison of traits heritabilities between populations revealed significantly lower heritabilities of masses in the *Robinia* larvae, whether reared on the control or tannin supplemented diet, suggesting that the ongoing process of adaptation to unfavorable nutrition (due to effects of selection) in the *Robinia* population reduced the expression of genetic variability of the larval mass. The significant genetic variability we recorded for most of the examined traits in gypsy moth larvae from both populations on the control and the stressful diet, allows the expression of a set of phenotypes and suggests their ability to adapt through responses to selection.

Plasticity of life-history traits is a mechanism that allows rapid and adequate responses of populations to environmental changes. Heritable variation of the norm of reaction slope, i.e., “genotype x environment” interaction, and the effects of selection on the norm of reaction slopes, lead to microevolutionary changes of phenotypic plasticity (Lynch and Walsh, 1998). The significant variability of phenotypic plasticity we recorded for most examined traits of gypsy moth larvae from both populations suggest that they are able to adapt phenotypically within one generation to a new/stressful environment. This points to the potential of gypsy moth, an insect generalist, to respond adaptively to selection in heterogeneous environments, and also to the potential for evolution of the phenotypic plasticity of more traits due to selective pressure of a specific environment.

The effects of inter-environmental genetic correlation represent an important influence on the rate and direction of evolution in traits related to the use of environmental resources (Via, 1984). The significant positive genetic correlations between duration of larval stages expressed in the two diets, recorded in both populations, suggest that families with short larval duration traits will also have short duration on a tannin supplemented diet. For larval masses, signifi-

cant correlations between environments were shown in the *Quercus*, but not in the *Robinia* populations. Correlations between environments for the traits are often non-significant, but they would move toward negative values in populations that become adapted to multiple hosts (Joshi and Thompson, 1995). The absence of negative genetic correlations may also mean that evolution of the norm of reaction is still in progress (Via and Lande, 1985). Although significant genetic correlations between environments (control and stressful diet) were positive, most of them were significantly different from “one” and do not represent a constraint for the evolution of plasticity.

The significant genetic variability and variability of phenotypic plasticity we recorded for the fitness-related traits in gypsy moth larvae from differently adapted populations, expressed in response to nutritive stress, points to their ability for adaptation and the potential for the evolution of phenotypic plasticity of the examined traits, as well as to the capacity for further expansion and broadening of ecological niches.

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