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ANALYSIS OF CORRELATION STRUCTURE IN *LYMANTRIA DISPAR* L. LARVAE FROM LOCALLY ADAPTED POPULATIONS

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Abstract - The influence of allelochemical stress and population origin on the patterns of phenotypic and genetic correlations among life history traits and digestive enzyme activities were investigated in larvae of the gypsy moth (*Lymantria dispar* L.; Lepidoptera: Lymantriidae). 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 an artificial diet, with or without a 5% tannic acid supplement. Comparison of correlation matrices revealed significant similarity between the two populations in the structure of phenotypic and genetic correlations of life history traits and of digestive enzyme activities. The patterns of correlations of the examined traits, within each of the two locally adapted populations and in the presence of allelochemical stress, remained stabile despite the different selection pressures that mold these traits.

Key words: Lymantria dispar, life history traits, digestive enzymes, patterns of correlations

INTRODUCTION

Phytophagous insects can use numerous host plant species across their geographic distribution, but within a local population, their feeding is usually restricted to one or a few plant species. If host plant species impose different selective pressures on insects, host-associated local adaptation may occur (Ruiz-Montoya et al., 2003). Different environmental conditions may induce different patterns of correlations among traits (Pigliucci and Kolodynska, 2002). Directional selection is expected to alter genetic correlations (Lande, 1979). Comparisons of correlation matrices can facilitate the identification of evolutionary constraints and developmental/functional relationships among traits (Baker and Wilkinson, 2003).

The gypsy moth is a composite generalist whose individual diet-breadth is narrower than the population diet-breadth (Rossiter, 1987). Local differentiation for some fitness, behavioral and physiological traits has been shown in gypsy moth larvae from populations with different host-use history (Perić Mataruga et al., 1997; Lazarević et al., 2002; Lazarević et al., 2003; Mrdaković et al. in press). Gypsy moth larvae from two populations with different trophic adaptations, originating from oak (suitable host plant) and locust-tree (unsuitable host plant) forests were analyzed. Although black locust is described as an unsuitable host plant for gypsy moth development (Barbosa and Krischik, 1987), our gypsy moth population has persisted in the locust-tree forest for more than 50 years, i.e. generations. In this forest, defoliation never occurs, i.e. population dynamics oscillate with smaller amplitude than in an oak forest (Sidor and Jodal, 1983). Both oak and locust-tree leaves are characterized by the presence of condensed and hydrolyzable tannins (Schultz and Baldwin, 1982; Rakesh et al., 2000), although the tannin content is higher in the locust-tree leaves (Unruh Snyder et al., 2007). Locust-tree leaves also contain some other allelochemicals (Nasir et al., 2005; Veitch et al., 2010), and have a higher buffering capacity and lower nutrient content, compared to oak leaves (Montgomery, 1986; Barbosa and Krischik, 1987). Gypsy moth larvae from both populations were exposed to the allelochemical stress, tannic acid in the rearing diet. Our investigations revealed significant influences of tannic acid on some of the life history traits and digestive enzyme activities of gypsy moth larvae from these populations (Mrdaković et al., 2011; Mrdaković et al., in press).

Different selective pressures that previous generations of gypsy moth from the two populations have experienced (to consume oak or locust-tree leaves), as well as the presence of stressors, could cause changes in correlation structures. The aim of our study was to examine the dependence of phenotypic and genetic correlation structures on the population origin and on the presence of an allelochemical stressor (tannic acid) in the rearing diet in gypsy moth larvae from different populations.

MATERIALS AND METHODS

As previously described (Mrdaković et al., 2011), one of the populations of *L. dispar* used in this study, referred to as the *Quercus* population, was collected from a mixed oak forest (predominantly *Quercus cerris* L.) within the Bogovadja forest complex, (locality “Bogovadja”, 70 km south-west of Belgrade). Thirty-two egg masses were randomly sampled from oak trees. The second population was collected from a man-made locust tree forest, referred to as the *Robinia* population, (locality “Bagremara”, 122 km north-west of Belgrade). Twenty-six egg masses were randomly sampled from black locust trees. The forest complex “Bagremara” is almost completely covered by black locust trees with a very few black walnut and English oak trees. The egg masses were kept in a refrigerator at 4°C until May, when they were transferred to a constant temperature of 23°C to hatch. They were reared at 23°C and a L12:D12 photoperiod. After molting to the fourth instar, the larvae were reared individually in Petri dishes (9cm diameter), and were provided daily with fresh food. From both populations, 7-9 larvae from each egg mass (unbalanced full-sib design) were randomly assigned to a group fed on the control diet, the high wheat-germ based (HWG) standard laboratory diet of the gypsy moth (O’Dell et al., 1985), and a group fed on the HWG diet containing 5% tannic acid (Tannic Acid, Sigma-Aldrich). Accordingly, there were four experimental groups: *Quercus* larvae fed on the control diet (QC), *Quercus* larvae fed on the tannin supplemented diet (QT), *Robinia* larvae fed on the control diet (RC) and *Robinia* larvae fed on the tannin supplemented diet (RT).

Examined traits

The following traits were measured for each larva: duration of development from hatching to molting into the 4th instar, duration of the 4th instar, duration of development from molting to hatching into the 5th instar; mass after molting into the 4th instar, mass after molting into the 5th instar, and mass on the 3rd day after molting into the 5th instar; relative growth rate from 3rd till 4th molt, and relative growth rate from 4th molt till 3rd day after molting into the 5th instar. Relative growth rates were calculated according to Giertych et al. (2005).

The relative midgut mass (RMM) (Jindra and Sehnal, 1989) and specific activities of digestive enzymes were also measured for each larva. The crude midgut extracts were used for the determination of the specific activity of digestive enzymes: total proteases (Kunitz, 1947), trypsin and leucine aminopeptidase (Erlanger et al., 1961), α-glucosidase (Baker, 1991), and lipase (Mrdaković et al., 2008).

Statistical methods

In order to estimate differences in the patterns of correlation of examined traits of larvae originating from different populations and reared on the different diets, Mantel's test (Mantel, 1967) (program NTSYSpc, Numerical Taxonomy System, Version 2.2 for Windows NT/2000/XP and Vista) was applied. The method involves a suitable correlation calculated between the corresponding elements of two matrices. The statistical significance of this matrix correlation was determined by a randomization process that produces a distribution of matrix correlation values, with 2000 permutations for each distribution. The null hypothesis of the test is that compared matrices have no similarity (Manly, 1994). Mantel's test is widely used to assess patterns of genetic distances, particularly for testing the statistical significance of genetic and geographic distance between correlation matrices (Smouse et al., 1986; Rodrigues et al., 2002), which is important for the assessment of patterns of population differences caused by different microevolutionary processes (Rodrigues and Diniz-Filho, 1998).

RESULTS

Statistically highly significant values of correlation coefficients were obtained in comparing the phenotypic, genetic and, phenotypic and genetic correlation matrices of larval life history traits of *L. dispar*, between the diets within each of the populations (QC-QT; RC-RT), and between the two populations on each of the diets (QC-RC; QT-RT) (Table 1). The original and randomized matrices did not differentiate significantly, which is not in agreement with the null hypothesis of the test. Comparisons of phenotypic and genetic matrices within each of analyzed group revealed substantial similarity (Table 1).

Comparison of the structure of phenotypic correlations between life history traits on one side and RMM and digestive enzyme activities on the other side in the *Robinia* larvae revealed a significant negative correlation coefficient among matrices on the control and tannin supplemented diet (RC-RT). In addition, by comparison of the genetic correlation matrices of these traits, we noticed significant negative correlation coefficients between the two diets within the *Quercus* population (QC-QT), and between the *Quercus* and *Robinia* larvae reared on the control diet (QC-RC), (Table 2). Comparison of genetic correlation matrices of life history traits and RMM and digestive enzyme activities between the two diets within the *Robinia* population, revealed a correlation coefficient close to zero. The obtained values suggest significant differences of correlation structures between the examined groups. Other Mantel correlation coefficients were statistically significant, indicating the similarity of the matrices. Comparisons of phenotypic and genetic correlation matrices within each of the analyzed groups revealed their similarity (Table 2).

By comparison of genetic correlation matrices among RMM and digestive enzyme specific activities between the two populations on the control diet (QC-RC), (Table 3), we noticed that there was no similarity between these matrices, i.e. Mantel's correlation coefficient was not significant (P>0.05). Other correlation coefficients obtained by Mantel's test for the comparison of the phenotypic and genetic correlation matrices of RMM and digestive enzymes activities between the two populations on each of the diets, and between the diets in each population, were statistically significant, indicating a similarity of correlation patterns. Comparisons of the phenotypic matrices with the matrices of genetic correlations within each of the analyzed groups also revealed substantial similarity (Table 3).

DISCUSSION

The gypsy moth, *Lymantria dispar* L., whose diet breadth is estimated at more than 500 plant species, is a serious defoliator of Palearctic and Nearctic forests (Lance, 1983). Adaptation to different environmental conditions within a certain habitat, which leads to the formation of distinct local geographic populations, has been shown in European gypsy moth (Reineke et al., 1999).

In Serbia, gypsy moth is the most detrimental forest insect species with periodical outbreaks. Oak species are suitable host plants, and *Q. cerris* is the

Table 1. Correlation coefficients (r_M) in Mantel test for comparisons of phenotypic and genetic correlation matrices of life history traits between different treatments within *Quercus* (QC-QT) and *Robinia* (RC-RT) populations, between populations on each of the diet (QC-RC), (QT-RT), and comparison of genetic and phenotypic correlation matrices within each experimental group (QC, QT, RC, RT).

	r_M	P
Phenotypic		
QC-QT	0.975	0.0005
RC-RT	0.941	0.0005
QC-RC	0.919	0.0005
QT-RT	0.958	0.0005
Genetic		
QC-QT	0.930	0.0005
RC-RT	0.819	0.0005
QC-RC	0.760	0.0005
QT-RT	0.729	0.0015
Phenotypic-genetic		
QC	0.967	0.0005
QT	0.933	0.0005
RC	0.904	0.0005
RT	0.857	0.0010

Table 2. Correlation coefficients (r_M) in Mantel test for comparisons of phenotypic and genetic correlation matrices of life history traits and RMM and specific enzyme activities between different treatments within *Quercus* (QC-QT) and *Robinia* (RC-RT) populations, between populations on each of the diet (QC-RC), (QT-RT), and comparison of genetic and phenotypic correlation matrices within each experimental group (QC, QT, RC, RT).

	r_M	P
Phenotypic		
QC-QT	0.427	0.0005
RC-RT	-0.025	0
QC-RC	0.427	0.0005
QT-RT	0.481	0.0005
Genetic		
QC-QT	-0.162	0
RC-RT	0.015	0
QC-RC	-0.076	0
QT-RT	0.236	0.0215
Phenotypic-genetic		
QC	0.594	0.0005
QT	0.830	0.0005
RC	0.682	0.0005
RT	0.802	0.0005

Table 3. Correlation coefficients (r_M) in Mantel test for comparisons of phenotypic and genetic correlation matrices of RMM and specific enzyme activities between different treatments within *Quercus* (QC-QT) and *Robinia* (RC-RT) populations, between populations on each of the diet (QC-RC), (QT-RT), and comparison of genetic and phenotypic correlation matrices within each experimental group (QC, QT, RC, RT).

	r_M	P
Phenotypic		
QC-QT	0.928	0.0050
RC-RT	0.959	0.0005
QC-RC	0.966	0.0020
QT-RT	0.932	0.0075
Genetic		
QC-QT	0.681	0.0335
RC-RT	0.799	0.0005
QC-RC	0.586	0.0640
QT-RT	0.802	0.0005
Phenotypic-genetic		
QC	0.895	0.0005
QT	0.983	0.0005
RC	0.772	0.0005
RT	0.921	0.0015

most favorable for gypsy moth development. The locust-tree is an unfavorable host plant as its leaves contain higher quantities of various allelochemicals and is of poorer nutritional quality than oak leaves. Previous studies revealed between-population differences in larval traits in gypsy moths with different host-use history. On average, a significantly higher longevity and pupal mass is shown in gypsy moth from the locust-tree than from the oak forest. Significant between-population variation in phenotypic plasticity in response to feeding on locust-tree leaves is recorded for preadult viability, duration of pupal stage, and for relative growth rate and gross growth efficiency of fourth gypsy moth instar (Lazarević et al., 2002; 2007). Perić Mataruga et al. (2001, 2011) describe differences in number and activity of protocerebral neurosecretory neurons in gypsy moth larvae from locust-tree and oak forest populations exposed to trophic stress. Regardless of exposure to stress, the constitutive activity of antioxidative enzymes is higher in the midgut tissue of locust-tree than of oak larvae. Our previous results concerning enzyme activities (Mrdaković et al., in press) also suggest between-population differences and the local adaptation of gypsy moth populations from oak and locust-tree forests. However, the different selective pressures experienced by previous generations of gypsy moth from the two populations, as well as the presence of the stressor (5% tannic acid in the rearing diet) did not cause significant changes in correlation structures. The results of Mantel's test mainly showed no significant differences in the patterns of correlations of examined traits between the two locally adapted gypsy moth populations. However, by comparing matrices of genetic correlations of life history traits and RMM and specific enzyme activities, we noticed differences within both populations, likely generated by the stressful effect of tannic acid. The difference in the structure of genetic correlations between the populations on the control diet could indicate the adaptation of *Robinia* larvae, possibly at the level of enzyme activities, to the presence of stressors. Correlations between life history traits and the activities of enzymes are important for determining the possibility for selection on enzyme activity, and might provide evidence for trade-offs between enzyme activity and other aspects of performance (Berrigan and Hoang, 1999).

Knowing the differences in patterns of correlations between populations can provide an opportunity for examining the balance between the local selection pressures (imposed by host plant specific defense) on larvae from different populations, and the effect of high gene flow. High gene flow between gypsy moth populations is promoted by the characteristic behavior – high dispersal and migration ability (Doane and McManus, 1981), as well as, its population dynamics. Phenotypic plasticity has an important role in promoting diversification but may also dampen the effects of natural selection by allowing rapid phenotypic adaptation of individuals to new conditions (Crispo, 2008). The plasticity of an insect related to its host allows the production of an optimal phenotype without major genetic changes (Via, 1990). The patterns of correlations of life history traits as well as patterns of correlations of enzyme activities, within each of the populations and in the presence of allelochemical stress, did not change despite the different selection pressures that are likely affecting these traits. The significant phenotypic plasticity expressed in response to stressful conditions that we earlier described in gypsy moth from both oak and locust-tree populations (Lazarević et al., 2002; Mrdaković et al., 2011), suggests their similar strategies for overcoming stressful conditions and achieving optimal phenotypes.

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