Heterozygosity Maintains Developmental Stability of Sternopleural Bristles in Drosophila subobscura Interpopulation Hybrids

Authors: Novicic, Zorana Kurbalija, Stamenkovic-Radak, Marina, Pertoldi, Cino, Jelic, Mihailo, Veselinovic, Marija Savic, et al.

Heterozygosity maintains developmental stability of sternopleural bristles in *Drosophila subobscura* interpopulation hybrids

Zorana Kurbalija Novicic[1a]*, Marina Stamenkovic-Radak[1,2], Cino Pertoldi[3,4], Mihailo Jelic[2], Marija Savic Veselinovic[2], Marko Andjelkovic[1,2]

[1]Institute of Biological Research, University of Belgrade, Despot Stefan Blvd. 142, 11000 Belgrade, Serbia
[2]Faculty of Biology, University of Belgrade, Studentski trg 3, 11000 Belgrade, Serbia
[3]Department of Ecology and Genetics, Institute of Biological Science, University of Aarhus, Ny Munkegade, Building 540, DK8000 Aarhus C, Denmark
[4]Mammal Research Institute, Polish Academy of Sciences, 17-230 Białowieża, Poland

Abstract

Interpopulation hybridization can lead to outbreeding depression within affected populations due to breakdown of coadapted gene complexes or heterosis in hybrid populations. One of the principal methods commonly used to estimate the level of developmental instability (DI) is fluctuating asymmetry (FA). We used three genetically differentiated *Drosophila subobscura* populations according to inversion polymorphism analysis and measured the variability of sternopleural bristle number and change in FA across generations P, F1, and F2 between intra- and interpopulation hybrids of *D. subobscura*. The mean variability of sternopleural bristle number in intra- and interpopulation hybrids of *D. subobscura* across generations cannot determine whether the changes at the level of developmental homeostasis are due exclusively to genomic coadaptation or to heterozygosity. Phenotypic variance (V_p) and FA of sternopleural bristle number was higher in interpopulation than in intrapopulation hybrids across generations. F1 hybrids were more developmentally stable compared to each parental population in both intra- and interpopulation hybrids. The most probable mechanism providing developmental homeostasis is heterozygote or hybrid superiority, also called overdominace. However, V_p was higher and FA lower in the F2 generation when compared to F1, due mainly to crossing-over in the formation of F2.

> **Cite this paper as:**
> Novicic ZK, Stamenkovic-Radak M, Pertoldi C, Jelic M, Veselinovic MS, Andjelkovic M. 2011. Heterozygosity maintains developmental stability of sternopleural bristles in *Drosophila subobscura* interpopulation hybrids. *Journal of Insect Science* 11:113 available online: insectscience.org/11.113

Introduction

Hybrid zones occur when two genetically distinct populations meet, mate, and produce offspring of mixed ancestry (Barton and Hewitt 1985; Ross and Robertson 1990). These zones can occur in disrupted habitats through interpopulation hybridization, where previously isolated populations become intermixed and hybridize. Anthropogenic activities in natural ecosystems may increase opportunities for interpopulation hybridization through habitat disruption. Hybridization between individuals from different populations can lead to heterosis, also called overdominace, in the first generation, followed by outbreeding depression in the consecutive generation due to the breakdown of coadapted gene complexes (Dobzhansky 1950; Andersen et al. 2002; Edmands 2007). There is growing evidence that environmental and genomic stressors may induce significant levels of developmental instability (DI) (Palmer and Strobeck 1986; Palmer 1996; Møller and Swaddle 1997; Pertoldi et al. 2006a; Kurbalija et al. 2010), suggesting that DI could serve as an early signal in monitoring the effects of stress in populations (Leary and Allendorf 1989).

Developmental stability is the ability of an organism to buffer environmental and genetic disturbances which affect the developmental capacity of a particular phenotype (Zakharov 1981; Palmer 1996). The failure to correct for random accidents during development may be manifested as fluctuating asymmetry (FA), which is defined by small deviations from perfect bilateral symmetry of morphological structures (Leary and Allendorf 1989; Palmer and Strobeck 1986).

The genetic basis of developmental stability has been much debated over the last five decades. The increase or decrease of DI as a consequence of the genomic stress has been explained by two major hypotheses. The first argues that the level of stability is a reflection of the underlying genomic heterozygosity (Lerner 1954), while the second argues that stability reflects the general level of genomic coadaptation (Dobzhansky 1950).

The coadaptive gene complexes are established over the evolutionary history of the genome via natural selection and are defined as a specific balance between loci within the genome (Dobzhansky 1950). Such coordination within the genome protects individuals from developmental accidents, which can be caused by both environmental and genetic factors (Parsons 1990). A breakdown of coadaptation can be manifested in an individual as a decreased ability to develop an optimal phenotype due to increased DI (Leary et al. 1985; Markow 1995).

Heterozygosity theory predicts that the level of heterozygosity is correlated with DI (Lerner 1954; Livshits and Kobyliansky 1985), such that heterozygous individuals are better adapted to genomic and environmental perturbations than homozygous individuals due to higher developmental stability. The theory also states that single loci coding for enzymes related to metabolic efficiency influence developmental stability of different morphometric characters (Palmer 1996; Møller and Swadle 1997), predicting that levels of heterozygosity at loci coding for functional proteins will be inversely correlated with level of DI (Lerner 1954; Livshits and Kobyliansky 1985). However, an important assumption is that heterozygosity of various genetic markers accurately reflects the heterozygosity of the entire genome, or at

least the heterozygosity of the loci that contribute to the formation of the morphological phenotype (Mitton and Grant 1984; Chakraborty 1987).

Whether heterozygosity and/or genomic coadaptation influence parameters of developmental stability is still unclear (Pertoldi et al. 2006b). Available data indicate a tendency of FA to increase with inbreeding (Palmer and Strobeck 1986; Waldmann 1999, 2001; Babbitt 2006; Fessehaye et al. 2007), hybridization between species (Hochwender and Fritz 1999; Stamenkovic-Radak et al. 2009), or between populations (Waldmann 1999; Kurbalija et al. 2010), although several studies report exceptions to this pattern (Clarke et al. 1992; Sheridan and Pomiankowski 1997; Trotta et al. 2005). A consensus does appear to exist in evidence that any stress would increase phenotypic variance (V_p) of most quantitative traits (Bijlsma and Loeschcke 1997; Hoffmann and Parsons 1997; Hoffmann and Hercus 2000; Pertoldi et al. 2001a,b).

It is well known among *Drosophila* spp. that V_p of individuals collected in the field is greater than those reared in a laboratory setting (Coyne and Beecham 1987; Imasheva et al. 1994; David et al. 1997; Gibert et al. 1998; Gibert et al. 2004). The higher phenotypic variability in flies from the wild is generally attributed to higher environmental variance, resulting in lower heritability (Coyne and Beecham 1987; Gibert et al. 1998; Hoffmann and Hercus 2000). It has been suggested that several mechanisms can alter the components of V_p (Waddington 1942), and recent studies have aimed to identify their respective roles within an evolutionary context (e.g. Zakharov 1992; Debat and David 2001; Meiklejohn and Hartl 2002).

Sternopleural bristle number is a widely studied characteristic in quantitative genetics (Mackay 2004) that displays a rapid response to artificial selection in *Drosophila* spp. (Ramuson 1955; Barnes and Kearsey 1970; Schnee and Thompson 1984). The genetic variance of SB obtained from IF analysis in *Drosophila melanogaster* was higher at extreme temperatures (Imasheva et al. 1998; Pétavy et al. 2006). Some studies show a positive correlation between FA of sternopleural bristle number and temperature variations (Parsons 1961; Imasheva et al. 1997; Bubliy et al. 2000; Pétavy et al. 2006) and nutritional variations as stress conditions (Imasheva et al. 1999). The results of these studies support the rather popular point of view that environmental stress is associated with higher FA levels (Møller and Swaddle 1997). On the contrary, Woods et al. (1999) reported that stress did not induce an increase in the V_p or FA of sternopleural bristle number in *D. melanogaster*. Despite heterogeneity among results in the association between different kinds of environmental stresses and FA and V_p as estimators of DI, evidence is scarce regarding the effects of interspecies or interpopulation hybridization as a genomic stressor and its correlation with FA of this meristic trait in *Drosophila* species (Gilligan et al. 2000; Andersen et al. 2002; Vishalakshi and Singh 2008).

Inversion polymorphism of different *Drosophila* species was used as a model system for studying processes involved in adaptation and genetic diversity (Balanya et al. 2004; Hoffmann et al. 2004; Stamenkovic-Radak et al. 2008). As crossing-over is suppressed within the inversion loops of heterokaryotypes, all genes within the inverted segments segregate as a linked group representing one physical and functional unit,

called the supergene, the different arrangements of which can be referred to as allelic complexes (Krimbas 1993; Rasic et al. 2008; Santos 2009). *D. subobscura* is a Palearctic species that displays rich inversion polymorphism in all five acrocentric chromosomes of the set (Krimbas and Loukas 1980; Krimbas 1992, 1993). Assuming a relatively long-time of selection on the linked genes within inverted regions, *D. subobscura* represents a suitable species for testing the heteozygosity vs. coadaptation hypotheses.

This paper focuses on the coadaptive aspect of inversion polymorphism in *D. subobscura* populations from three ecologically and topologically distinct habitats, which possess a certain degree of genetic differentiation due to their different evolutionary histories. The aim of the study is to investigate the level of DI estimated by FA and V_p in sternopleural bristle (SB) number in intra- and interpopulation hybrids through 3 generations (P, F1 and F2) in isofemale lines (IF) of *D. subobsura*. The study intends to contribute to understanding the effects of coadaptations and heterozygosity on the level of developmental homeostasis in intra- and interpopulation hybrids.

Materials and Methods

Population samples

For the present study, *D. subobscura* individuals were sampled in Serbia using fermented fruit traps. The flies were collected from three localities (beech-B, oak-O and Botanical Garden-BG). The first locality, Beech wood (B) (*Abieto-fagetum*), is situated between N 43°33' 28.43" and E 20°45' 10.96" (Mountain Goc, Central Serbia). The second is Oak wood (O) (*Fraxineto-quercetum*) situated between N 43°32' 57.38" and E 20°40' 2.32" (Mountain Goc, Central Serbia).

These two woods have distinctive microclimates; Beech wood has the highest altitude (875 m) and the highest humidity with great vegetation coverage, while Oak wood (787 m) has more sparsely distributed trees and is slightly warmer. The third locality is the Botanical Garden at the University of Belgrade (BG) (*Arboretum-Corilus colurna, Celtis australis*), situated between N 44° 49' and E 20 ° 28' at 87 m above sea level, representing a more urbanized environment with a unique microclimate and high anthropogenic influence.

The females collected at these three localities were individually used to obtain isofemale lines (IF) reared on the common cornmeal-sugar-yeast-agar medium for *Drosophila*. The progeny of these IF lines were used as the parental generation in the experiment in order to preserve the high amount of genetic variability from natural population. All cultures were maintained and all experiments performed under constant laboratory conditions at 19°C, approximately 60% relative humidity, light of 300 lux and 12:12 L:D cycles.

Population genetic structure and data analysis

Analysis of inversion polymorphism was carried out for the wild captured *D. subobscura* males, which were individually crossed with virgin females from Küsnacht laboratory stock, homozygous for Standard gene arrangement at all five large chromosomes. Salivary glands from third-instar larvae were squashed and chromosomes stained with aceto-orcein solution. Eight larvae were analyzed from the progeny of each of the crosses performed. The chromosome map from Kunze-Muhl and Muller (1958) was used for the cytological analysis of gene arrangements. The

designation of gene arrangements followed that of Kunze-Muhl and Sperlich (1955). The analysis included in total 56 males (112 autosomes, 56 sex chromosomes) from Oak population, 44 males from Beech population (88 autosomes, 44 sex chromosomes), and 52 males from Botanical Garden (104 autosomes, 52 sex chromosomes).

Z-statistics (Zar 1999) were used to assess the differences between frequencies of gene arrangements in the pairs of analyzed populations. The G-test (Sokal and Rohlf 1980) was used to determine population subdivision by determining the homogeneity of gene arrangement frequencies between pairs of populations from different forest communities on all chromosomes and autosomes.

Experimental design

The experiment used 63 IF lines from Oak, 38 from Beech, and 64 from the Botanical Garden; the progeny of these IF lines were used as the parental (P) generation in the experiment. Virgin males and females were separated within each IF line upon emerging and intra- and interpopulation crosses were made four days after eclosion.

Intrapopulation (B × B, O × O, BG × BG) and interpopulation crosses (B × O, BG × O, BG × B) were made among IF lines of the three *D. subobscura* populations. Both direct and reciprocal crosses were made in order to take into account any maternal effect (Table 1). The progeny (six males, six females) from each cross were transferred to fresh vials to obtain F1 and F2 generations. Individuals in P, F1, and F2 generations from intra- and interpopulation crosses (B × B, O × O, BG × BG, B × O, BG × O, BG × B) were frozen at −20ºC and used for further sternopleural bristles counting procedure.

Count of sternopleural bristle number

The left and right side of the sternopleural region from each fly was observed under a 100× binocular microscope and the SB number was scored and counted twice, first on the right side (R), then on the left side (L). This meristic characteristics and others may be measured without error thus avoiding the need for replicate measurements (Palmer 1994). According to most other similar investigations, all short, medium-sized, and long bristles were counted.

Fluctuating asymmetry data analyses

Before interpreting FA population estimates, several statistical assumptions were made. In the folowing statistical analysis, males and females from each population, each generation, and direct and reciprocal crosses were analyzed separately. Because of the large number of tests conducted, the sequential Bonferroni test (Rice 1989) was used. Fluctuating asymmetry is characterized by normal distribution of the right-side minus left-side differences with a mean of zero and a normal distribution (Palmer and Strobeck 1986). No deviations from normal distribution was found using Shapiro-Wilk (W) and Chi-squared (χ^2) tests.. One-sample *t*-tests were performed to test for a departure from the mean of (R − L) from the expected mean of zero; no directional asymmetry was detected in all tested samples.Tests for correlation between SB number and FA were done because significant correlation may affect results and interpretation (Palmer 1994; Palmer and Strobeck 2003). Linear dependence of FA on the mean SB number was tested by linear regression; FA acted as the explanatory variable and SB number as the dependent variable. In 43.05% cases we found significant positive corelation between FA and SB number (Table 2).

Because higher variance was typically observed in cases where more bristles were found, a log transformation was used on variance values and further tests were done with transformed values to account for a scaling effect of the mean. The FA1 index (Palmer 1994) was measured as $|R - L|$ between sides in all samples of intrapopulation and interpopulation hybrids, both direct and reciprocal crosses, separately for males and females through P, F1, and F2 generations. The FA1 index is the one of the most frequently used indices for describing the level of FA in a sample. It also acts as an unbiased estimator of the sample standard deviation, and is recommended for testing FA differences between three or more samples (Palmer and Strobeck 1992). However, because of the significant presence of positive correlation between FA and SB number, we also used FA2 index (Palmer 1994). The FA2 index (mean ($|R - L|/((R+L)/2)$)) is commonly used only where clear evidence exists of a size dependence of $|R - L|$ among individuals within a sample.

The multivariate ANCOVA was conducted to determine effects of intra- and interpopulation hybridization, generation, sex as an independent variable, and direct and reciprocal crosses as covariate variables while controlling for FA1, FA2, and mean sternopleural bristle number, the latter acting as a dependent variable. The analysis was performed using general multivariate linear model in SPSS software.

Student t-tests and F-tests were used to test differences in mean and variances of the sternopleural bristle number and FA between sexes, populations, generations, and type of cross. All tests treated sex, population, generation, and type of cross as separate variables. The conservative F-test and t-test were used to reduce the possibility of a Type 1 error. Furthermore, we have performed corrections for multiple comparisons and therefore our results are both conservative and robust, and not affected by eventual interactions between the different factors. All statistical analyses were performed using PAST software (Hammer et al. 2001).

Results

Population genetic structure

Frequencies of gene arrangements and parameters of inversion polymorphism (Degree of Heterozygosity, Inversion Density, Index of Free Recombination) on five acrocentric chromosomes of *D. subobscura* population from three different natural habitats are shown in Table 3. Inversion polymorphism analysis of Beech population (B) detected 15 stuctural chromosomal types and 13 inversions; in the Oak (O) and the Botanical garden populations (BG), 16 stuctural chromosomal types and 15 inversions were detected.

Results of the G-test gave the level of interpopulation differences in gene arrangement distribution for each individual chromosome. The results showed significant differences in gene arrangement distribution between B and O populations for four chromosomes: A (G = 10.91, $p < 0.01$), J (G = 14.49, $p < 0.01$), U (G = 11.10, $p < 0.01$), and E (G = 9.52, $p < 0.05$), and for all chromosomes in total (G = 49.24, $p < 0.01$) (Table 4).

Interpopulation differences in gene arrangement distributions of each chromosome were found between B and BG populations: U (G = 9.70, $p < 0.01$), E (G = 12.84, $p < 0.01$), O (G = 14.44, $p < 0.01$), and

for all chromosomes in total (G = 39.25, $p < 0.01$) (Table 4).

Interpopulation differences in gene arrangement distributions on each chromosome were found between O and BG populations: J (G = 17.02, $p < 0.01$), U (G = 30.44, $p < 0.01$), E (G = 31.23, $p < 0.01$), O (G = 24.53, $p < 0.01$), and for all chromosomes in total (G = 116.95, $p < 0.01$) (Table 4).

The results of the Z-test showed the level of interpopulation differences in individual gene arrangement frequencies and revealed significant differences in some gene arrangement frequencies between each pair of tested populations (B and O, B and BG, O and BG) (Table 5).

The results showed significant differences in some gene arrangement frequencies for all chromosomes of the set between some populations (B and O, BG and O). There were significant differences in gene arrangement frequencies between B and BG populations, for U, E and O chromosomes (Table 5). Gene arrangement $E_{1+2+9+12}$ was detected only in O population, as well as gene arrangement O_6 which was detected only in BG population with a frequency of less than 3%.

Multivariate testing of fluctuating asymmetry and mean of SB number

The results of the multivariate test using a general linear model are presented in Table 6. Significant differences were detected between populations; intrapopulation and interpopulation hybrids (F = 29.524, $p < 0.01$), generations (F = 113.114, $p < 0.01$), and type of cross (F = 2.995, $p < 0.05$). Significant interactions were also found between population × generation (F = 59.919,

$p < 0.01$) and generation × sex (F = 11.022, $p < 0.01$).

The results test of between-subject effect are shown in Table 7. The significant differences were found between populations (FA1, F = 22.471, $p < 0.01$; FA2, F = 20.153, $p < 0.01$; mean SB number, F = 19.895, $p < 0.01$), generations (FA1, F = 49.513, $p < 0.01$; FA2, F = 85.854, $p < 0.01$; mean SB number, F = 65.351, $p < 0.01$). Significant interaction was also found between population × generation (FA1, F = 15.901, $p < 0.01$; FA2, F = 13.197, $p < 0.01$; mean SB number, F = 44.140, $p < 0.01$).

Univariate testing of fluctuating asymmetry and mean of SB number
Intrapopulation hybridization.
Changes of mean and variance across generations in males. Analysis of the difference in mean and variance of the average SB number in males is given in Table 8a. Generally, very low variability in sternopleural bristle number in *D. subobscura* populations was observed. A significant increase of the average SB number was detected through generations (P < F1 < F2) in O × O hybrids resulting from direct cross. In B × B hybrids, a significant increase in SB number was detected in the F1 generation when compared to the parental generation, followed by a decrease in SB number in the F2 generation in both direct and reciprocal crosses.

In BG × BG hybrids, a trend of constant increase in SB number was detected across generations (P < F1 < F2) in both direct and reciprocal crosses.

No significant change in variance across generations was detected except in the case of BG × BG hybrids for both direct ($F_{P,F1} = 1.58$,

$p < 0.05$; $F_{F1,F2} = 1.45$, $p < 0.01$) and reciprocal crosses ($F_{P,F1} = 1.69$, $p < 0.05$; $F_{F1,F2} = 1.32$, $p < 0.05$).

Changes of mean and variance across generations in females. Analysis of the difference in mean and variance of the average SB number in females is given in Table 8b. The statistically significant increase of SB number was successive through generations for all hybrid groups in both direct and reciprocal crosses. Regarding the variance, no statistically significant change was detected across generations in all tested groups of intrapopulation hybrids for females.

Changes of FA across generations in males. Analysis of the FA1 index between generations for SB number in males shows no significant difference between generations in the direct cross. There was, however, significant decrease of FA value across generations in O × O (t $_{P,F1}$ = 3.48, $p < 0.01$; t $_{P,F2}$ = 4.00, $p < 0.01$) and B × B (t $_{F1,F2}$ = 2.41, $p < 0.05$) hybrids in reciprocal crosses (Table 9a).

Analysis of the FA2 index between generations for SB number in males shows no significant difference between generations in the direct cross, although there was significant decrease of FA value across generations in O × O (t $_{P,F1}$ = 2.84, $p < 0.01$; t $_{P,F2}$ = 3.66, $p < 0.01$) hybrids in reciprocal crosses (Table 8a).

Changes of FA across generations in females. Analysis of FA differences between generations using FA1 index for SB number in females shows no significant difference between generations in both direct and reciprocal crosses except in the case of B × B hybrids (t $_{P,F1}$ = 2.03, $p < 0.05$) in direct cross and O × O hybrids (t $_{F1,F2}$ = 3.38, $p < 0.01$) in reciprocal cross (Table 9b).

Analysis of FA differences between generations using FA2 index for SB number in females shows no significant difference between generations in both direct and reciprocal crosses, except in the case of O × O hybrids in reciprocal cross, where FA value significantly decreased across generations (t $_{F1,F2}$ = 3.69, $p < 0.01$). In the case of B × B hybrids, a constant decrease of FA across generations was detected (t $_{P,F2}$ = 2.60, $p < 0.05$; t $_{F1,F2}$ = 2.41, $p < 0.05$) (Table 10b).

Inter-population hybridization
Changes of mean and variance across generations in males. Analysis of the difference in mean and variance of the average SB number in males is given in Table 8a. The results showed significant differences in SB number and variance in most interpopulation hybrids groups in both direct and reciprocal crosses. In B × O hybrids an increase in SB number in F1 generation was detected when compared to the parental generation, followed by a significant decrease in the F2 generation (t $_{F1,F2}$ = 2.35, $p < 0.05$) in direct crosses. A different trend was found for reciprocal crosses (P > F1 > F2). In the case of BG × O hybrids, the significant increase in average SB number across generations was detected (P < F1 < F2) in direct crosses. In BG × B hybrids, a significant decrease of SB number in the F1 generation was found when compared to the parental generation, followed by a significant increase in SB number in the F2 generation for both direct and reciprocal crosses.

The variance in SB number in B × O hybrids showed a statistically significant increase in F2 compared to F1 generation in both direct (F $_{F1,F2}$ = 1.83, $p < 0.01$) and reciprocal crosses (F $_{F1,F2}$ = 1.16, $p < 0.01$). In the case of BG × O hybrids, the variance showed a

significant constant increase across generations (P < F1 < F2) for both direct and reciprocal crosses. In the BG × B hybrids a significant increase of variance was detected in F2 when compared to F1 generation in reciprocal crosses ($F_{P,F2} = 1.82$, $p < 0.05$; $F_{F1,F2} = 1.61$, $p < 0.01$).

Changes of mean and variance across generations in females. Analysis of the difference in mean and variance of the average SB number in females is given in Table 8b. In B × O hybrids an increase of average SB number was detected in the F1 generation when compared to the parental generation followed by a decrease in the F2 generation in reciprocal crosses. In BG × O hybrids a statistically significant increase of SB number was detected across generations (P < F1 < F2) in both direct and reciprocal crosses. In BG × B hybrids a significant decrease in average SB number was detected in the F1 generation when compared to the parental generation, and an increase in the same value for the F2 generation (P > F1 < F2) in both direct and reciprocal crosses

A decrease in variance was detected in the F1 generation followed by a significant increase in the F2 generation for B × O hybrids in both direct ($F_{P,F2} = 1.71$, $p < 0.05$; $F_{F1,F2} = 2.03$, $p < 0.01$) and reciprocal crosses ($F_{P,F2} = 2.73$, $p < 0.01$; $F_{F1,F2} = 2.93$, $p < 0.01$). In BG × O and BG × B hybrids a significant increase of variance was detected in the F2 generation when compared to the F1 generation in both direct and reciprocal crosses.

Changes of FA across generations in males. Analyses of the FA1 index between generations for sternopleural bristles in males are given in Table 9a. The results showed a statistically significant decrease in FA values across generations in B × O hybrids in both

direct ($t_{P,F2} = 4.50$, $p < 0.01$; $t_{F1,F2} = 6.03$, $p < 0.01$) and reciprocal crosses ($t_{P,F2} = 4.80$, $p < 0.01$; $t_{F1,F2} = 7.07$, $p < 0.01$). In BG × O hybrids a significant decrease of FA was detected in the F2 generation when compared to the F1 generation in both direct ($t_{F1,F2} = 2.19$, $p < 0.05$) and reciprocal crosses ($t_{F1,F2} = 2.86$, $p < 0.01$). In BG × B hybrids a significant increase of FA was detected across generations in both direct ($t_{P,F2} = 2.57$, $p < 0.05$) and reciprocal crosses ($t_{P,F1} = 2.44$, $p < 0.05$).

Analyses of the FA2 index between generations for SB number in males are given in Table 10a. The results showed a statistically significant decrease of FA values across generations in B × O hybrids in both direct ($t_{P,F2} = 5.41$, $p < 0.01$; $t_{F1,F2} = 6.44$, $p < 0.01$) and reciprocal crosses ($t_{P,F2} = 4.67$, $p < 0.01$; $t_{F1,F2} = 7.50$, $p < 0.01$). In BG × O hybrids a decrease of FA values was detected in the F2 generation when compared to the F1 generation in reciprocal crosses ($t_{F1,F2} = 3.17$, $p < 0.05$). In BG × B hybrids a decrease of FA values was detected across generations in reciprocal crosses ($t_{P,F1} = 2.22$, $p < 0.05$; $t_{P,F2} = 2.60$, $p < 0.01$).

Changes of FA across generations in females. The results of FA1 analyses between generations for SB number in females are given in Table 9b. The results showed a significant increase in FA for B × O hybrids in the F2 generation when compared to the F1 generation in both direct ($t_{F1,F2} = 7.16$, $p < 0.01$) and reciprocal crosses ($t_{F1,F2} = 6.59$, $p < 0.01$). In BG × O hybrids a significant decrease of FA was found in reciprocal crosses ($t_{F1,F2} = 3.19$, $p < 0.01$). In BG × B hybrids a significant decrease of FA was found across generations in direct crosses ($t_{P,F2} = 2.57$, $p < 0.05$).

The results of FA2 analyses between generations for SB number in females are given in Table 10b. The results showed an increase in FA value in the F1 generation when compared to the parental generation, along with a significant decrease in the F2 generation for B × O hybrids (P < F1 > F2) in both direct and reciprocal crosses. In BG × O hybrids a significant decrease of FA was found in the F2 generation when compared to the F1 generation in direct crosses (t $_{F1,F2}$ = 3.08, $p < 0.01$), while a statistically significant decrease in FA across generations was found in reciprocal crosses (t $_{F1,F2}$ = 2.63, $p < 0.01$;t $_{F1,F2}$ = 3.68, $p < 0.01$). In BG × B hybrids a statistically significant decrease of FA was detected across generations in direct crosses (t $_{P,F2}$ = 3.10, $p < 0.01$; t $_{F1,F2}$ = 3.58, $p < 0.01$). The same trend of FA variability across generations was also found in reciprocal crosses (t $_{F1,F2}$ = 1.97, $p < 0.001$).

Discussion

The results showed that populations of *D. subobscura* from three ecologically and topologically distinct habitats possess a certain degree of genetic difference, likely due to their different evolutionary histories. The gene arrangements are carriers of various alleles that are differently favored in diverse environmental conditions and prove in most cases to be the major factor determining the gene arrangement frequencies in natural populations of *D. subobscura* (Andjelkovic et al. 2003). The results presented here further emphasize the relationship between gene arrangement frequencies and local adaptation to ecologically different microhabitats in *D. subobscura*.

The inversion polymorphism analysis showed that analysed populations differ in the frequencies of some gene arrangements that are in agreement with previous results (Andjelkovic et al. 2003; Stamenkovic-Radak et al. 2008; Jelic et al. 2009). In general, the pattern of inversion polymorphism of the three analyzed *D. subobscura* populations was consistent with the observed inversion polymorphism of *D. subobscura* in the southeast margin of the Central European region (Krimbas 1993). Therefore, the results suggest that the three populations in this study are suitable for testing the coadaptation vs. heterozygosity hypothesis.

This paper focuses on the coadaptive aspect of genetic variability at the population level and its relationship to interpopulation hybridization as a genomic stressor. The trait used in the study was a typical meristic character widely considered as a threshold trait (Falconer and Mackay 1996; Mackay 2001, 2004). Some data suggest the existence of particular genes that affect the genotype × environmental covariance of SB number (Kristensen et al. 2005; Sørensen et al. 2007). The underlying continuous variable is both genetic and environmental in origin and could be measured and studied equally as a metric character but with greater precision because of non-existence of measurement error.

The results regarding the mean of SB number for intra- and interpopulation hybrids of *D. subobscura* cannot explicitly reveal the significance of either of the two hypotheses, since no general trend has emerged for either intra- and interpopulation hybrids. However, the observed variance of the SB number across generations gives a completely different insight.

The variance of SB number after intra- and interpopulation hybridization showed that intrapopulation hybrids have no significant change of variance across generations in most

of the hybrid groups, compared to interpopulation hybrids. However, a significant change of variance across generations is observed in interpopulation hybrids. A reduction of V_p in the F1 generation compared to the parental generation was obtained in most of the crosses, signifying that the hybrids had reached a higher level of homeostasis in the F1 generation, likely due to higher heterozygosity. Also, a significantly higher level of V_p in the F2 generation was obtained when compared to the F1 generation, probably due to disruption of coadapted gene complexes, which can be attributed to crossing over in formation of most of the genotypes in the F2 generation.

The analysis of the variability of FA of SB number using FA1 and FA2 indices (Palmer 1994) after intra- and interpopulation hybridizations showed that intrapopulations hybrids are relatively stable. Analysis showed no significant differences in FA in most of the cases between generations when compared to the interpopulation hybrids across generations. This result was expected when the effects of intrapopulation crosses are taken into account, which simulate random mating in natural populations. These crosses represent control conditions for genomic stress. On the other hand a significant trend of change in FA was observed across generations in interpopulation hybrids, which was not observed in intrapopulation hybrids.

Theoretically, a heterosis in the F1 generation and outbreeding depression caused by disruption of coadaptive gene complexes in the F2 generation for interpopulation hybrids was expected, but results showed a post-hybridization decrease in FA for interpopulation hybrids in both F1 and F2 generations.

The results showed a significant decrease of FA through generations each with the lowest FA detected in the F2 generation in all interpopulation hybrids. Therefore, it is likely that the observed difference between intra- and interpopulation hybrids regarding level of FA variability is due to increased heterozygosity in the F1 generation in interpopulation hybrids, and thus the increased developmental homeostasis and reduced FA. An increase of heterozygosity also reduced the FA in the F2 generation, which could be a result of selection pressure acting on F2 genotypes. Selection could eliminate developmentally unstable and extreme phenotypes from the population at very early stage and overall hybrid fitness could increase (Polak et al. 2002). The increase of FA in the F2 generation compared to the F1 generation was not obtained, indicating the possibility that disruption of coadaptation occurred and the less stable genotypes produced by crossing over were selected against. Therefore, in the F2 generation, we obtained only individuals with suitable gene combinations and higher developmental stability. This suggests that the most probable mechanism providing developmental homeostasis in a population is the heterozygote or hybride superiority, also known as overdominance.

Heterosis can be considered the opposite of inbreeding depression; both mechanisms share the same underlying causes (Crow 1993). The primary cause of heterosis is the sheltering of deleterious recessive alleles in hybrids. In addition, increased heterozygosity would increase the fitness of hybrid individuals at loci where heterozygotes have a selective advantage over homozygote types. A subdivision of natural populations can provide an appropriate condition for heterosis, as

different deleterious recessive alleles could drift to relately high frequencies in different subpopulations. Therefore interpopulation hybrids produced by mating between immigrant individuals are expected to have greater fitness than residents (Whitlock et al. 2000; Morgan 2002).

The evidence for heterosis without coadaptation in *Drosophila ananassae* was presented in Sing (1972, 1985), showing that interracial hybridization does not lead to breakdown of heterosis associated with cosmopolitan inversions. These results provide observations that may carry weight on testing heterozygosity vs. coadaptation , but the findings of heterosis in interpopulation hybrids does not exclude hybrid breakdown in subsequent generations (Lynch and Walsh 1997).

In response to genomic stress, no significant difference was found between the majority of direct and reciprocal crosses, suggesting that parental population origin had no significance and that both sexes equally contribute to the inheritance of SB number. This suggests that sternopleural bristles, as a bilaterally symmetrical morphological trait, might be a good and consistent characteristic for measuring the level of developmental stability under genomic stress without a confounding maternal effect. These results contrast with wing FA in a previous study (Kurbalija et al. 2010).

There may be several explanations for the observed difference in the response of different bilateral symmetrical traits to genomic stress conditions. The development of distinct traits is probably under control of different gene complexes (Parsons 1990). A stress factor may also be specific for particular metabolic pathways and may not affect the FA

of all traits in the same way (Palmer and Strobeck 1986). Furthermore, different traits are under different natural selection pressure that depends on functional importance of a given trait. Sternopleural bristles are not generally regarded as a fitness-related trait and we do not expect them to be a highly canalized trait.

Despite the fact that different outcomes can be expected after interpopulation hybridization, depending on evolutionary histories of populations and trait specific response, our results confirmed that DI could be a useful indicator of genomic stress in populations. Further knowledge on the consequences of interpopulation hybridization between wild populations as well as the capacity of small populations to adapt to local environmental conditions is urgently needed.

The integration of experimental, theoretical, and applied conservation genetics will contribute to understanding of the methodological and applied aspects of conservation genetics. It seems that *Drosophila* is a useful species to bridge the gap between theoretical computer simulation and studies on natural populations.

Acknowledgements

References

Andersen DH, Pertoldi C, Scali V, Loeschcke V. 2002. Intraspecific hybridisation, developmental stability and fitness in *Drosophila mercatorum. Evolutionary Ecology Research* 4:603-621.

Andjelkovic M, Savkovic V, Kalajdzic P. 2003. Inversion polymorphism in *Drosophila subobscura* from two different habitats from the mountain of Goc. *Hereditas* 138: 241-243.

Babbitt GA. 2006. Inbreeding reduces power-law scaling in the distribution of fluctuating asymmetry: an explanation of the basis of developmental instability. *Heredity* 97: 258-268.

Balanyà J, Solé E, Oller JM, Sperlich D, Serra L. 2004. Long-term changes in the chromosomal inversion polymorphism of *Drosophila subobscura.* II. European populations. *Journal of Zoological Systematics and Evolutionary Research* 42: 191-201.

Barton NH, Hewitt GM. 1985. Analysis of hybrid zones. *Annual Review of Ecology and Systematics* 16: 113-148.

Barnes BW, Kearsey MJ. 1970. Variation for metrical characters in *Drosophila* populations. I. Genetics analysis. *Heredity* 25: 1-10.

Bijlsma R, Loeschcke V. 1997. *Environmental Stress, Adaptation and Evolution*. Birkhäuser Verlag.

Bubliy OA, Loeschcke V, Imasheva AG. 2000. Effects of stressful and nonstressful growth temperatures on variation of sternopleural bristle number in *Drosophila melanogaster. Evolution* 54: 1444-1449.

Chakraborty R. 1987. Biochemical heterozygosity and phenotypic variability of polygenic traits. *Heredity* 59: 19-28.

Clarke GM, Oldroyd BP, Hunt P. 1992. The genetic basis of developmental stability in *Apis mellifera*: Heterozygosity versus genetic balance. *Evolution* 46**: 7**53-762.

Coyne J, Beecham E. 1987. Heritability of two morphological characters within and among natural populations of *Drosophila melanogaster. Genetics* 117: 727-737.

Crow JF. 1993. Mutation, mean fitness and, genetic load. *Oxford Surveys in Evolutionary Biology* 9: 3-42.

David JR, Gibert P, Gravot E, Pétavy G, Morin JP, Karan D. 1997. Phenotypic plasticity and developmental temperature in *Drosophila*: analysis and significance of reaction norms of morphometrical traits. *Journal of Thermal Biology* 22: 441-451.

Debat V, David P. 2001. Mapping phenotypes: canalization, plasticity and developmental stability. *Trends in Ecology and Evolution* 16: 555-561.

Dobzhansky T. 1950. Genetics of natural populations. XIX. Origin of heterosis through natural selection in populations of Drosophila pseudoobscura. *Genetics* 35: 288-302.

Edmands S. 2007. Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Molecular Ecology* 16: 463-475.

Falconer DS, Mackay TFC .1996. Introduction to Quantitative Genetics, 4th. Edition. Pearson Education Limited.

Fessehaye Y, Komen H, Rezk MA, van Arendonk JAM, Bovenhuis H. 2007. Effects of inbreeding on survival, body weight and fluctuating asymmetry (FA) in Nile tilapia, *Oreochromis niloticus. Aquaculture* 264: 27-35.

Gibert P, Moreteau B, Moreteau JC, David JR. 1998. Genetic variability of quantitative traits in *Drosophila melanogaster* (fruit fly) natural populations: analysis of wild-living flies and of several laboratory generations. *Heredity* 80: 326-335.

Gibert P, Capy P, Imasheva A, Moreteau B, Morin JP, Pétavy G. 2004. Comparative analysis of morphometrical traits among *Drosophila melanogaster* and *D. simulans*: genetic variability, clines and phenotypic plasticity. *Genetica* 120: 165-179.

Gilligan DM. 2000. Can fluctuating asymmetry be used to detect inbreeding and loss of genetic diversity in endangered populations? *Animal Conservation* 3: 97-104.

Hammer Ø, Harper D, Ryan PD. 2001. PAST: Paleontological statistics software package for education and data analysis. *Paleontologia Electronica* 4:9.

Hochwender CG, Fritz RS. 1999. Fluctuating asymmetry in a *Salix* hybrid system: the importance of genetic versus environmental causes. *Evolution* 53: 408-416.

Hoffmann AA, Parsons PA. 1997. Extreme Environmental Change and Evolution. Cambridge University Press.

Hoffmann AA, Hercus MJ. 2000. Environmental stress as an evolutionary force. *Bioscience* 50: 217-226.

Hoffmann AA, Sgrò CM, Weeks AR. 2004. Chromosomal inversion polymorphisms and adaptation. *Trends in Ecology and Evolution* 19: 482-488.

Imasheva AG, Bubli OA, Lazebny OE. 1994. Variation in wing length in Eurasian populations of *Drosophila melanogaster. Heredity* 72: 508-514.

Imasheva AG, Loeschcke V, Zhivotovsky LA, Lazebny OE. 1997. Effects of extreme temperatures of phenotipic variation and developmental stability in *Drosophila melanogaster* and *Drosophila buzzatii*. *Biological Journal of Linnean Society* 61: 117-126.

Imasheva AG, Loeschcke V, Zhivotovsky LA, Lazebny OE. 1998. Stress temperatures and quantitative variation in *Drosophila melanogaster*. *Heredity* 81: 246-253.

Imasheva AG, Bosenko DV, Bubli OA. 1999. Variation in morphological traits of *Drosophila melanogaster* (fruit fly) under nutritional stress. *Heredity* 3: 187-192.

Jelic M, Kenig B, Kurbalija Z, Stamenkovic-Radak M, Andjelkovic M. 2009. Intra-species differentiation among *Drosophila subobscura* from different habitats in Serbia. *Archives of Biological Science* 61: 513-521.

Krimbas CB, Loukas M. 1980. The inversion polymorphism of *Drosophila subobscura*. *BMC Evolutionary Biology* 12: 163-234.

Krimbas CB. 1992. The inversion polymorphism of *Drosophila subobscura*. In:

Krimbas CB, Powell JR, Editors. *Drosophila inversion polymorphism*. pp 127-220. CRC Press.

Krimbas CB. 1993. *Drosophila subobscura*: Biology, Genetics, and Inversion Polymorphism. Kovac.

Kristensen TN, Sørensen AC, Sorensen D, Pedersen KS, Sørensen JG, Loeschcke V. 2005. A test of quantitative genetic theory using Drosophila-effect of inbreeding and rate of inbreeding on heritabilities and variance components. *Journal of Evolutionary Biology* 18: 763-770.

Kurbalija Z, Stamenkovic-Radak M, Pertoldi C, Andjelkovic M. 2010. Outbreeding causes developmental instability in *Drosophila subobscura. Evolutionary Ecology* 24: 839-864.

Kunze-Muhl E, Sperlich D. 1955. Inversionen und chromosomale Strukturtypen bei *Drosophila subobscura* Coll. Zeitchsrift Induktive Abstammungs-u. *Vererbungslehre* 87: 65-84.

Kunze-Muhl E, Muller E. 1958. Weitere Untersuchungen über die chromosomale Struktur und natürlichen Strukturtypen von *Drosophila subobscura. Chromosoma* Berlin 9: 559-570.

Lerner IM. 1954. *Genetic Homeostasis*. John Wiley and Sons.

Livshits G, Kobliansky E. 1985. Lerner's concept of developmental homeostasis and the problem of heterozygosity level in natural populations. *Heredity* 55: 341-353.

Leary RF, Allendorf FW. 1989. Fluctuating asymmetry as an indicator of stress: implications for conservation biology. *Trends in Ecology and Evolution* 4: 214-217.

Leary RF, Allendorf FW, Knudsen KL. 1985. Developmental instability and high meristic counts in interspecific hibrids of salmonide fishes. *Evolution* 39: 1318-1326.

Lynch M, Walsh B. 1997. Genetics and Analysis of quantitative Traits. Sinauer Associates.

Markow TA. 1995. Evolutionary ecology and developmental instability. *Annual Review of Entomology* 40: 105-120.

Mackay TFC. 2001. The genetics architecture of quantitative traits. *Annual Review of Genetics* 35: 303-339.

Mackay TFC. 2004. The genetic architecture of quantitative traits: lessons from Drosophila. *Current Opinion in Genetics and Development* 14: 253-257.

Meiklejohn CD, Hartl DL. 2002. A single mode of canalization. *Trends in Ecology and Evolution* 17: 468-473.

Mitton JB, Grant MC. 1984. Association among protein heterozygosity, growth rate, and developmental homeostasis. *Annual Review of Ecology and Systematics* 15: 479-499.

Morgan MT. 2002. Genome-wide deleterious mutations favours dispersal and species integrity. *Heredity* 89: 253-257.

Møller AP, Swaddle JP. 1997. Asymmetry, developmental stability, and evolution. Oxford University Press.

Palmer AR, Strobeck C. 1986. Fluctuating asymmetry: measurement, analysis, patterns. *Annual Review of Ecology and Systematics* 17: 391-421.

Palmer AR, Strobeck C. 1992. Fluctuating asymmetry as a measure of developmental stability: implications of non-normal distributions and power of statistical tests. *Acta Zoologici Fennici* 191: 55–70.

Palmer AR. 1994. Fluctuating asymmetry analyses: a primer. In: Markow TA, Editor. *Developmental instability: its origins and evolutionary implications*. pp. 335-364. Springer Publishing Company.

Palmer AR. 1996. Waltzing with asymmetry: is fluctuating asymmetry a powerful new tool for biologists or just an alluring new dance step? *BioScience* 46: 518-532.

Palmer AR, Strobeck C. 2003. Fluctuating Asymmetry Analyses Revised. In: Polak M, Editor. *Developmental Instability: Causes and Consequences.* pp. 279-319. Oxford University Press.

Parsons PA. 1961. Fly size, emergence time and sternopleural cheta number in Drosophila. *Heredity* 16: 455-473.

Parsons PA. 1990. Fluctuating asymmetry: an epigenetic measure of stress. *Biological Review* 65: 131-145.

Pertoldi C, Loeschcke V, Scali V. 2001a. Developmental stability in sexually reproducing and parthenogenetic populations of *Bacillus rossius rossius* and *Bacillus rossius redtenbachery*. *Evolutionary Ecology Research* 4: 449-463.

Pertoldi C, Kristensen TN, Loeschcke V. 2001b. A new method for estimating environmental variability for parthenogenetic organisms, and the use of fluctuating asymmetry as an indicator of developmental stability. *Journal of Theoretical Biology* 4: 407-410.

Pertoldi C, Kristensen TN, Andersen DH, Loeschcke V. 2006a. Developmental instability as an estimator of genetic stress. *Heredity* 96: 122-127.

Pertoldi C, Sørensen JG, David JR, Loeschcke V. 2006b. Lerner's theory on the genetic relationship between heterozigosity, genomic co-adaptations, and developmental instability revised. *Evolutionary Ecology Research* 8: 1487-1498.

Pétavy G, David JR, Debat V, Pertoldi C, Moreteau B. 2006. Phenotypic and genetic variability of sternopleural bristle number in *Drosophila melanogaster* under daily thermal stress: developmental instability and anti-asymmetry. *Evolutionary Ecology Research* 8: 149-167.

Polak M, Opoka R, Cartwright IL. 2002. Response of fluctuating asymmetry to arsenic toxicity: support for the developmental selection hypothesis. *Environmental Pollution* 118: 19-28.

Ramuson M. 1955. Selection on bristle numbers in some unrelated strains of *Drosophila melanogaster. Acta Zoologica* 36: 7-49.

Rasic G, Stamenkovic-Radak M, Savic T, Andjelkovic M. 2008. Inbreeding reveals interpopulation differences in inversion polymorphism of *Drosophila subobscura*.

Journal of Zoological Systematics and Evolutionary Research 46: 31-37.

Rice WR. 1989. Analysing tables of statistical tests. *Evolution* 43: 223–225.

Ross KG, Robertson JL. 1990. Developmental stability, heterozygosity, and fitness in two introduced fire ants (*Solenopsis invicta* and *S. richteri*) and their hybrids. *Heredity* 64: 93-103.

Santos M. 2009. Recombination Load in a Chromosomal Inversion Polymorphism of *Drosophila subobscura. Genetics* 181: 803–809.

Schnee FB, Thompson JN. 1984. Conditional polygenic effects in the sternopleural bristle system of *Drosophila melanogaster*. *Genetics* 108: 409-424.

Singh BN. 1972. The lack of evidence for coadaptation in geographic population of *Drosophila ananassae*. *Genetica* 57: 139-142.

Singh BN. 1985. Heterosis without selection adaptation in *Drosophila ananassae. Theoretical and Applied Genetics* 69: 437-441.

Sheridan L, Pomiankowski A. 1997. Fluctuating asymmetry, spot asymmetry and inbreeding depression in the sexual coloration of male guppy fish. *Heredity* **79**: 515-523.

Sokal RR, Rohlf FJ. 1980. Biometry, 3rd Edition. WH Freeman and Company.

Sørensen AC, Kristensen TN, Loeschcke V, Ibañes N, Sorensen D. 2007. Genetically controlled environmental variance for sternopleural bristles in *Drosophila melanogaster-* an experimental test of

heterogeneous variance model. *Acta Agriculturae Scandinavica* 57: 196-201.

SPSS Incorporated Staff. 1999. SPSS base 10.0 for Windows user guide. Prentice Hall Professional Technical Reference.

Stamenkovic-Radak M, Rasic G, Savic T, Kalajdzic P, Kurbalija Z, Kenig B, Andjelkovic M. 2008. Monitoring of the genetic structure of natural populations: change of the effective population size and inversion polymorphism in *Drosophila subobscura*. *Genetica* 133: 57-63.

Stamenkovic-Radak M, Kostic I, Rasic G, Junakovic N, Andjelkovic M. 2009. Developmental stability of interspecies hybrids among *Drosophila melanogaster*, *D. simulans* and *D. mauritiana* (Diptera: Drosophilidae). *Acta Entomologica Serbica* 14: 27-37.

Trotta V, Calboli FCF, Garola F, Grifoni D, Cavicchi S. 2005. Fluctuating asymmetry as a measure of ecological stress in *Drosophila melanogaster* (Diptera: Drosophilidae). *European Journal of Entomology* 102: 195-200.

Vishalakshi C, Singh BN. 2008. Fluctuating Asymmetry in Hybrids of Sibling Species, *Drosophila ananassae* and *Drosophila pallidosa*, Is Trait and Sex Specific. *Journal of Heredity* 100: 181-191.

Waddington CH. 1942. Canalization of development and the inheritance of acquired characters. *Nature* 150: 563-565.

Waldmann P. 1999. The effect of inbreeding and population hybridization on developmental instability in petals and leaves

of the rare plant *Silene diclinis* (Caryophyllaceae). *Heredity* 83: 138-144.

Waldmann P. 2001. The effect of inbreeding on fluctuating asymmetry in *Scabiosa canescens* (Dipsacaceae). *Evolutionary Ecology* 15: 117-127.

Whitlock MC, Ingvarsson PK, Hatfield K. 2000. Local drift load and heterosis of interconnected populations. *Heredity* 84: 452-457.

Woods RE, Sgro CM, Hercus MJ and.Hoffmann AA. 1999. The association between fluctuating asymmetry, trait variability, trait heritability, and stress: a multiply replicated experiment on combined stresses in *Drosophila melanogaster*. *Evolution* 53: 493-505.

Zakharov VM. 1981. Fluctuating asymmetry as an index of developmental homeostasis. *Genetica* 13: 241-256.

Zakharov VM. 1992. Population phenogenetics: analysis of developmental stability in natural populations. *Acta Zoologici Fennici* 191: 7-30.

Zar HJ. 1999. Biostatistical analysis, 4th Edition. Prentice-Hall.

Table 1. Number and type of intra- and inter-population crosses for direct and reciprocal crosses.

Type of cross	Direct cross	Reciprocal cross	Number of crosses
	Inter- isofemale line crosses		
Intra-population			
OxO	O♀ x O♂	O♂ x O♀	63
BxB	B♀ x B♂	B♂ x B♀	71
BGxBG	BG♀ x BG♂	BG♂ x BG♀	89
Inter-population			
BxO	B♀ x O♂	B♂ x O♀	82
BGxO	BG♀ x O♂	BG♂ x O♀	81
BGxB	BG♀ x B♂	BG♂ x B ♀	73

O, Oak population; B, Beech population; BG,Botanical Garden population

Table 2. Tests for linear dependence of FA on SB average number using linear regression, of intra- and inter-population crosses in P, F1 and F2 generations in males and females (direct and reciprocal crosses).

		Males						Females						
		direct			reciprocal			direct			reciprocal			
Cross	generation	N	correlation	p	N	correlation	p	N	correlation	p	N	correlation	p	
Intra-population														
	P	16	-0.19		16	0.637		16	0.018		16	0.124		
OxO	F1	73	0.367	**	71	-0.035		62	0.075		67	0.195		
	F2	173	0.263	***	132	0.223	*	199	0.234	***	167	0.117		
	P	13	0.217		13	0.533		13	0.321		13	-0.2		
BxB	F1	61	0.2		58	0.088		62	0.017		55	0.045		
	F2	115	0.094		116	0.112		144	0.205	**	138	0.097		
	P	49	0.073		49	0.169		49	0.175		49	0.126		
BGxBG	F1	215	0.147	*	222	0.167		207	0.309	***	208	0.188	***
	F2	593	0.18	***	615	0.099	*	712	0.176	***	686	0.217	***
Inter-population														
	P	42	-0.21		42	0.276		42	0.134		42	0.293		
BxO	F1	195	-0.005		186	0.051		192	-0.045		180	0.021		
	F2	433	0.361	***	410	0.432	***	518	0.311	***	493	0.28	***
	P	46	-0.053		46	0.106		46	0.299	*	46	0.145		
BGxO	F1	215	0.22	***	203	0.004		198	0.107		187	0.2	***
	F2	444	0.167	***	372	0.258	***	528	0.289	***	484	0.233	***
	P	44	0.132		44	0.23		44	0.421	**	44	0.344	*
BGxB	F1	208	0.092		215	0.23	***	202	0.096		190	0.204	**
	F2	582	0.096	*	582	0.189	***	673	0.248	***	648	0.204	

O, Oak population; B, Beech population; BG, Botanical garden population.
p<0.05 *, p<0.01**, p<0.001***

Table 3. Gene arrangement frequency (%) and inversion polymorphism parameters of *Drosophila subobscura* populations from three different habitats.

Gene arrangement	O (N=56)		B (N=44)		BG (N=52)	
	n	%	n	%	n	%
Ast	22	39.29%	29	65.91%	31	59.62%
A_1	32	57.14%	11	25.00%	19	36.54%
A_2	2	3.57%	4	9.09%	2	3.85%
J_{st}	3	2.68%	16	18.18%	20	19.23%
J_1	109	97.32%	72	81.82%	84	80.77%
U_{st}	8	7.14%	9	10.23%	24	23.08%
U_{1+2}	25	22.32%	37	42.05%	50	48.08%
U_{1+2+6}	79	70.54%	42	47.73%	30	28.85%
E_{st}	18	16.07%	15	17.05%	40	39.42%
E_8	17	15.18%	26	29.55%	30	28.85%
E_{1+2+9}	74	66.07%	47	53.41%	34	31.73%
$E_{1+2+9+12}$	3	2.68%	0	0.00%	0	0.00%
O_{st}	12	10.71%	9	10.23%	24	24.04%
O_6	0	0.00%	0	0.00%	2	0.96%
O_{3+4}	44	39.29%	45	51.14%	56	52.88%
O_{3+4+1}	47	41.96%	30	34.09%	16	15.38%
O_{3+4+2}	9	8.04%	4	4.55%	6	6.73%
HZ	1.64		2.48		2.17	
IFR	86.08		80.29		80.96	
ID	7.27		6.89		5.88	

O = Oak population, B = Beech population, BG =Botanical garden population
HZ, Degree of Heterozigosity; IFR, Index of Free Recombination; ID, Inversion Density
N = number of gene arrangement; N = number of analyzed males

Table 4. Chromosomal differences in arrangement frequencies between three analyzed habitats of *Drosophila subobscura* populations.

Chromosomes	B/O	B/BG	O/BG
A	10.91 **		
J	14.49 ***	0.03	17.02 ***
U	11.01 **	9.70 **	30.41 ***
E	9.52 *	12.84 **	31.23 ***
O		14 44 **	24.53 ***
all	49.24 ***	39.25 ***	116.95 ***

O = Oak, B = Beech ,BG = Botanical garden. p<0.05 *, p<0.01 **, p<0.001 ***
G-test values are given only for significant comparisons.

Table 5. Differences in gene arrangement frequencies between three analyzed habitats of *Drosophila subobscura* populations.

Gene arrangement	B/O	B/BG	O/BG
A_{st}	3.76 ***		(-)2.99 **
A_1	(-)4.59 ***		3.03 **
A_2			
J_{st}	5.29 ***		(-)5.58 ***
J_1	(-)5.29 ***		5.58 ***
U_{st}		(-)3.33 ***	(-)4.66 ***
U_{1+2}	4.26 ***		(-)5.62 ***
U_{1+2+6}	(-)4.66 ***	3.82 ***	8.66 ***
E_{st}		(-)4.64 ***	(-)5.25 ***
E_8	3.49 ***		(-)3.44 ***
E_{1+2+9}	(-)2.59 **	4.11 ***	6.94 ***
$E_{1+2+9+12}$	(-)2.20 *		2.38 *
O_{st}		(-)3.34 ***	(-)3.45
O_6			(-)2.09 *
O_{3+4}	2.38 *		(-)3.03 **
O_{3+4+1}		4.29 ***	6.08 ***
O_{3+4+2}			

O = Oak, B = Beech ,BG = Botanical garden. p<0.05 *, p<0.01 **, p<0.001 ***
Z-test values are given only for significant comparisons.

Table 6. Results of multivariate test using General linear model.

Effect		Value	F	Hypothesis df	Error df	Significance
Intercept	Pillai's Trace	0.731	13359.979a	3	14765	0
	Wilks' Lambda	0.269	13359.979a	3	14765	0
	Hotelling's Trace	2.715	13359.979a	3	14765	0
	Roy's Largest Root	2.715	13359.979a	3	14765	0
type of cross	Pillai's Trace	0.001	2.995a	3	14765	0.03
	Wilks' Lambda	0.999	2.995a	3	14765	0.03
	Hotelling's Trace	0.001	2.995a	3	14765	0.03
	Roy's Largest Root	0.001	2.995a	3	14765	0.03
population	Pillai's Trace	0.016	16.268	15	44301	0
	Wilks' Lambda	0.984	16.295	15	40760.07	0
	Hotelling's Trace	0.017	16.314	15	44291	0
	Roy's Largest Root	0.01	29.524b	5	14767	0
generation	Pillai's Trace	0.023	57.463	6	29532	0
	Wilks' Lambda	0.977	57.764a	6	29530	0
	Hotelling's Trace	0.024	58.064	6	29528	0
	Roy's Largest Root	0.023	113.114b	3	14766	0
sex	Pillai's Trace	0	1.636a	3	14765	0.179
	Wilks' Lambda	1	1.636a	3	14765	0.179
	Hotelling's Trace	0	1.636a	3	14765	0.179
	Roy's Largest Root	0	1.636a	3	14765	0.179
population x generation	Pillai's Trace	0.038	19.134	30	44301	0
	Wilks' Lambda	0.962	19.33	30	43338.87	0
	Hotelling's Trace	0.04	19.522	30	44291	0
	Roy's Largest Root	0.037	53.919b	10	14767	0
population x sex	Pillai's Trace	0.001	1.03	15	44301	0.42
	Wilks' Lambda	0.999	1.03	15	40760.07	0.42
	Hotelling's Trace	0.001	1.03	15	44291	0.42
	Roy's Largest Root	0.001	2.483b	5	14767	0.03
generation x sex	generation x sex	0.003	6.904	6	29532	0
	Wilks' Lambda	0.997	6.905a	6	29530	0
	Hotelling's Trace	0.003	6.906	6	29528	0
	Roy's Largest Root	0.002	11.022b	3	14766	0
population x generation x sex	Pillai's Trace	0.002	0.936	30	44301	0.566
	Wilks' Lambda	0.998	0.936	30	43338.87	0.566
	Hotelling's Trace	0.002	0.936	30	44291	0.566
	Roy's Largest Root	0.001	1.434b	10	14767	0.158

[a] Exact statistic; [b] The statistic is an upper bound on F that yields a lower bound on the significance level; [c] Design

Table 7. Results of tests of between-subjects effects using genelar linear model (multivariate).

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Significance
Corrected Model	FA1	245.883a	36	6.83	13.941	0
	FA2	6.661b	36	0.185	15.323	0
	(R+L)/2	1936.321c	36	53.787	46.089	0
Intercept	FA1	546.859	1	546.859	1116.192	0
	FA2	14.441	1	14.441	1195.89	0
	(R+L)/2	45229.161	1	45229.161	38756.33	0
type of cross	FA1	0.049	1	0.049	0.101	0.751
	FA2	0.007	1	0.007	0.574	0.449
	(R+L)/2	9.373	1	9.373	8.032	0.005
population	FA1	55.047	5	11.009	22.471	0
	FA2	1.217	5	0.243	20.153	0
	(R+L)/2	116.086	5	23.217	19.895	0
generation	FA1	48.516	2	24.258	49.513	0
	FA2	2.073	2	1.037	85.854	0
	(R+L)/2	152.532	2	76.266	65.351	0
sex	FA1	0.002	1	0.002	0.003	0.955
	FA2	0.003	1	0.003	0.219	0.64
	(R+L)/2	5.393	1	5.393	4.621	0.032
population x generation	FA1	77.905	10	7.79	15.901	0
	FA2	1.594	10	0.159	13.197	0
	(R+L)/2	515.117	10	51.512	44.14	0
population x sex	FA1	4.992	5	0.998	2.038	0.07
	FA2	0.11	5	0.022	1.828	0.104
	(R+L)/2	2.118	5	0.424	0.363	0.874
generation x sex	FA1	4.072	2	2.036	4.155	0.016
	FA2	0.07	2	0.035	2.882	0.056
	(R+L)/2	35.179	2	17.589	15.072	0
population x generation x sex	FA1	4.64	10	0.464	0.947	0.488
	FA2	0.137	10	0.014	1.136	0.33
	(R+L)/2	13.801	10	1.38	1.183	0.297
Error	FA1	7234.845	14767	0.49		
	FA2	178.322	14767	0.012		
	(R+L)/2	17233.29	14767	1.167		
Total	FA1	13775	14804			
	FA2	337.673	14804			
	(R+L)/2	623851.75	14804			
Corrected Total	FA1	7480.728	14803			
	FA2	184.983	14803			
	(R+L)/2	19169.611	14803			

[a] R Squared = .033 (Adjusted R Squared = .031); [b] R Squared = .036 (Adjusted R Squared = .034); [c] R Squared = .101 (Adjusted R Squared = .099)

Table 8a. The mean and variance (log transformed) for sternopleural bristle number in males of intra- and interpopulation crosses across generations (direct and reciprocal crosses).

cross	generation	N	mean±SE	t test	p	variance	F test	p	N	mean±SE	t test	p	variance	F test	p
				direct							**reciprocal**				
Intra-population															
	P	16	5.84±0.19	P<F1,t=-2.06	*	0.0039	P>F1,F=1.25		16	6.50±0.27	P>F1,t=1.29		0.004	P>F1,F=1.86	
OxO	F1	73	6.30±0.09	P<F2,t=-2.17	*	0.0031	P<F2,F=1.46		71	6.20±0.09	P>F2,t=0.97		0.0032	P>F2,F=1.24	
	F2	173	6.48±0.09	F1<F2,t=-1.19		0.0047	F1<F2, F=1.83		132	6.29±0.07	F1>F2,t=-0.81		0.0027	F1>F2,F=1.20	
	P	13	5.15±0.17	P<F1,t=-4.94	***	0.0031	P<F1,F=1.67		13	5.65±0.24	P<F1,t=-3.81	***	0.0048	P<F1,F=1.07	
BxB	F1	60	6.72±0.14	P<F2,t=-4.45	***	0.0051	P<F2,F=1.18		58	6.89±0.14	P<F2,t=-2.27	*	0.0051	P<F2,F=1.09	
	F2	115	6.19±0.07	F1>F2,t=3.58	***	0.0036	F1>F2,F=0.12		116	6.36±0.10	F1>F2,t=3.05	**	0.0052	F1<F2,F=1.03	
	P	49	5.76±0.13	P<F1,t=-3.05	**	0.0049	P>F1,F=1.58	*	49	5.86±0.14	P<F1,t=-2.36	*	0.0058	P>F1.F=1.69	*
BGxBG	F1	221	6.15±0.05	P<F2,t=-5.70	***	0.0031	P>F2,F=1.09		226	6.18±0.05	P<F2,t=-5.01	***	0.0034	P>F2,F=1.28	
	F2	593	6.64±0.04	F1<F2,t=-6.38	***	0.0045	F1<F2,F=1.45	**	615	6.66±0.04	F1<F2,t=-6.02	***	0.0045	F1<F2,F=1.32	*
Inter-population															
	P	42	5.77±0.13	P<F1,t=-1.39		0.0043	P>F1,F=1.44		42	6.33±0.24	P>F1,t=1.58		0.0037	P<F1,F=1.19	
BxO	F1	194	5.95±0.05	P>F2,t=0.11		0.003	P<F2,F=1.27		190	6.09±0.06	P>F2,t=4.31	***	0.0044	P<F2,F=1.39	
	F2	433	5.76±0.05	F1>F2,t=2.35	*	0.0055	F1<F2,F=1.83	***	409	5.67±0.05	F1>F2,t=5.11	***	0.0051	F1<F2,F=1.16	**
	P	46	5.97±0.10	P<F1,t=-3.05	**	0.0031	P<F1,F=1.21		46	6.06±0.10	P<F1,t=-2.21		0.0023	P<F1,F=1.94	
BGxO	F1	214	6.40±0.06	P<F2,t=-2.50	*	0.0037	P<F2,F=2.14	*	203	6.40±0.07	P>F1,t=-0.95		0.0044	P<F2,F=2.42	***
	F2	446	6.44±0.06	F1<F2,t=-0.38		0.0066	F1<F2,F=1.76	***	373	6.23±0.06	F1>F2,t=1.72		0.0055	F1<F2,F=1.24	*
	P	44	6.31±0.14	P>F1,t=3.41	***	0.0037	P<F1,F=1.17		44	6.39±0.13	P>F1,t=0.99		0.0034	P<F1,F=1.14	
BGxB	F1	207	5.82±0.06	P<F2,t=-1.06		0.0043	P<F2,F=1.33		215	6.25±0.06	P<F2,t=-1.13		0.0039	P<F2,F=1.82	*
	F2	584	6.49±0.05	F1<F2,t=-7.91	***	0.0049	F1<F2,F=1.14		586	6.61±0.05	F1<F2,t=-3.91	***	0.0063	F1<F2,F=1.61	***

O = Oak, B = Beech ,BG = Botanical garden. p<0.05 *, p<0.01 **, p<0.001 ***
The F test was used for testing differences in variances and t test were used for testing differences in means of SB number.

Table 8b. The mean and variance (log transformed) for sternopleural bristle number in females of intra- and interpopulation crosses across generations (direct and reciprocal crosses).

cross	generation	N	mean±SE	t test	p	variance	F test	p	N	mean±SE	t test	p	variance	F test	p
				direct							**reciprocal**				
Intra-population															
	P	16	6.19±0.16	P>F1,t=0.27		0.002	P<F1,F=1.62		16	6.03±0.22	P<F1,t=-1.26		0.0055	P>F1,F=1.72	
OxO	F1	62	6.13±0.10	P<F2,t=-1.10		0.0032	P<F2,F=2.30		67	6.32±0.09	P<F2,t=-2.47	*	0.0032	P>F2,F=1.57	
	F2	200	6.49±0.08	F1<F2,t=-2.43	*	0.0046	F1<F2, F=1.42		167	6.63±0.07	F1<F2,t=-2.41	*	0.0035	F1<F2,F=1.09	
	P	13	5.65±0.29	P<F1,t=-2.40	*	0.0073	P>F1,F=1.09		13	5.96±0.17	P<F1,t=-2.25	*	0.0021	P<F1,F=1.87	
BxB	F1	62	6.51±0.15	P<F2,t=-2.80	***	0.0067	P<F2,F=1.42		55	6.58±0.13	P<F2,t=-2.16	*	0.0039	P<F2,F=1.92	
	F2	145	6.62±0.10	F1<F2,t=-0.60	***	0.0051	F1>F2,F=1.30		138	6.55±0.08	F1>F2,t=0.18		0.0041	F1<F2,F=1.03	
	P	49	5.64±0.14	P<F1,t=-3.60	***	0.0062	P>F1,F=1.61		49	5.95±0.13	P<F1,t=-1.24		0.0051	P>F1,F=1.28	
BGxBG	F1	208	6.16±0.06	P<F2,t=-7.14	***	0.0038	P>F2,F=1.15		213	6.12±0.06	P<F2,t=-5.18	***	0.004	P>F2,F=1.05	
	F2	710	6.90±0.04	F1<F2,t=-8.21	***	0.0054	F1<F2,F=1.40		685	6.82±0.04	F1<F2,t=-8.12	***	0.0049	F1<F2,=1.22	
Inter-population															
	P	42	6.06±0.14	P>F1,t=0.34		0.0043	P>F1,F=1.18		42	6.08±0.13	P<F1,t=-0.68		0.0032	P>F1,F=1.07	
BxO	F1	192	6.01±0.60	P>F2,t=0.98		0.0036	P<F2,F=1.71	*	182	6.17±0.06	P>F2,t=1.47		0.003	P<F2,F=2.73	***
	F2	516	5.87±0.05	F1>F2,t=1.46		0.0073	F1<F2,F=2.03	***	493	5.81±0.05	F1>F2,t=3.88	***	0.0088	F1<F2,F=2.93	***
	P	46	6.19±0.10	P<F1,t=-0.59		0.0025	P>F1,F=1.51		46	6.02±0.10	P<F1,t=-2.27	*	0.0026	P<F1,F=1.33	
BGxO	F1	196	6.28±0.06	P<F2,t=-2.34	*	0.0038	P<F2,F=2.28	*	185	6.45±0.09	P<F2,t=-2.94	**	0.0035	P<F2,F=2.19	**
	F2	527	6.61±0.05	F1<F2,t=-3.57	***	0.0058	F1<F2,F=1.51	***	482	6.56±0.05	F1<F2,t=-1.00		0.0058	F1<F2,F=1.64	***
	P	44	6.35±0.10	P>F1,t= 2.30	*	0.0062	P>F1,F=1.61	*	44	6.35±0.11	P>F1,t=1.49		0.0027	P<F1,F=1.70	*
BGxB	F1	207	5.98±0.07	P<F2,t=-2.29	*	0.0038	P>F2,F=1.15		191	6.10±0.07	P<F2,t=-2.95	**	0.0047	P<F2,F=2.22	**
	F2	669	6.78±0.05	F1<F2,t=-8.50	***	0.0054	F1<F2,F=1.40	***	648	6.92±0.05	F1<F2,t=-8.17	***	0.0061	F1<F2,F=1.31	*

O = Oak, B = Beech ,BG = Botanical garden. p<0.05 *, p<0.01 **, p<0.001 ***
The F test was used for testing differences in variances and t test were used for testing differences in means of SB number.

Table 9a. The FA1 index differences between generations and type of crosses for sternopleural bristles in males (direct and reciprocal crosses); *t test* were used for testing differences in mean of SB.

				FA1= |R-L|					
			Direct				**Reciprocal**		
cross	generation	N	mean±SE	t test	p	N	mean±SE	t test	p
Intra-population									
	P	16	0.62±0.22	P>F1,t=0.28		16	1.12±0.22	P>F1,t=3.48	***
OxO	F1	73	0.57±0.07	P>F2,t=0.72		71	0.52±0.07	P>F2,t=4.00	***
	F2	173	0.49±0.05	F1>F2,t=0.91		132	0.45±0.05	F1>F2,t=-0.77	
	P	13	0.46±0.18	P<F1,t=-0.37		13	0.69±0.13	P>F1,t=0.36	
BxB	F1	60	0.53±0.08	P>F2,t=0.05		58	0.62±0.09	P>F2,t=1.94	
	F2	115	0.45±0.05	F1>F2,t=0.85		116	0.40±0.05	F1>F2,t=2.41	*
	P	49	0.51±0.10	P<F1,t=-0.31		49	0.45±0.12	P<F1,t=-1.72	
BGxBG	F1	221	0.54±0.03	P<F2,t=-0.77		226	0.62±0.04	P<F2,t=-1.49	
	F2	593	0.58±0.03	F1<F2,t=-0.95		615	0.60±0.03	F1>F2,t=0.48	
Inter-population									
	P	42	1.02±0.15	P>F1,t=1.35		42	1.00±0.13	P>F1,t=1.01	
BxO	F1	194	0.85±0.05	P>F2,t=4.50	***	190	0.87±0.05	P>F2,t=4.80	***
	F2	433	0.49±0.03	F1>F2,t=6.03	***	409	0.43±0.04	F1>F2,t=7.07	***
	P	46	0.85±0.11	P>F1,t=0.70		46	0.69±0.09	P<F1,t=-0.12	
BGxO	F1	214	0.77±0.05	P>F2,t=1.88		203	0.71±0.05	P>F2,t=1.50	
	F2	446	0.63±0.03	F1>F2,t=2.19	*	373	0.54±0.03	F1>F2,t=2.86	**
	P	44	1.07±0.14	P>F1,t=1.91		44	1.02±0.10	P>F1,t=2.44	*
BGxB	F1	207	0.83±0.05	P>F2,t=2.57	*	215	0.76±0.04	P>F2,t=1.71	
	F2	669	0.75±0.03	F1>F2,t=1.91		648	0.82±0.03	F1<F2,t=-0.98	

O = Oak, B = Beech ,BG = Botanical garden. p<0.05 *, p<0.01 **, p<0.001 ***

Table 9b. The FA1 index differences between generations and type of crosses for sternopleural bristles in females (direct and reciprocal crosses); *t test* were used for testing differences in means of SB.

		FA1= |R-L|							
		Direct				**Reciprocal**			
cross	generation	N	mean±SE	t test	p	N	mean±SE	t test	p
Intra-population									
	P	16	0.62±0.18	P<F1,t=-0.13		16	0.56±0.18	P<F1,t=-0.88	
OxO	F1	62	0.64±0.06	P>F2,t=0.21		67	0.70±0.06	P>F2,t=0.86	
	F2	200	0.59±0.04	F1>F2,t=0.62		167	0.43±0.04	F1>F2,t=3.38	***
	P	13	0.85±0.30	P>F1,t=1.07		13	0.38±0.24	P<F1,t=-0.97	
BxB	F1	62	0.63±0.07	P>F2,t=2.03	*	55	0.58±0.08	P<F2,t=-0.62	
	F2	145	0.45±0.05	F1>F2,t=1.95		138	0.51±0.06	F1>F2,t=0.62	
	P	49	0.47±0.10	P<F1,t=-1.99		49	0.53±0.10	P<F1,t=-0.89	
BGxBG	F1	208	0.67±0.04	P<F2,t=-1.64		213	0.61±0.04	P<F2,t=-1.21	
	F2	710	0.64±0.03	F1>F2,t=0.53		685	0.66±0.03	F1<F2,t=-0.86	
Inter-population									
	P	42	0.69±0.12	P<F1,t=-1.93		42	0.74±0.09	P<F1,t=-1.48	
BxO	F1	192	0.92±0.05	P>F2,t=1.76		182	0.91±0.05	P>F2,t=2.07	
	F2	516	0.48±0.03	F1>F2,t=7.16	***	493	0.50±0.03	F1>F2,t=6.59	***
	P	46	0.65±0.09	P<F1,t=-1.18		46	0.78±0.10	P>F1,t=0.09	
BGxO	F1	196	0.79±0.05	P<F2,t=-0.14		185	0.77±0.05	P>F2,t=1.85	
	F2	527	0.67±0.03	F1>F2,t=-1.18		482	0.58±0.03	F1>F2,t=3.19	**
	P	44	1.07±0.14	P>F1,t=1.91		44	0.84±0.11	P>F1,t=0.07	
BGxB	F1	207	0.83±0.05	P>F2,t=2.57	*	191	0.83±0.05	P>F2,t=0.20	
	F2	669	0.75±0.03	F1>F2,t=1.32		648	0.82±0.03	F1>F2,t=0.26	

O = Oak, B = Beech ,BG = Botanical garden. p<0.05 *, p<0.01 **, p<0.001 ***

Table 10a. The FA2 index differences between generations and type of crosses for sternopleural bristles in males (direct and reciprocal crosses); *t test* were used for testing differences in mean of SB.

		FA2=mean |R-L| /mean [(R+L)/2]							
		Direct				**Reciprocal**			
cross	generation	N	mean±SE	t test	p	N	mean±SE	t test	p
Intra-population									
	P	16	0.10±0.04	P>F1,t=0.56		16	0.16±0.03	P>F2,t=2.84	**
OxO	F1	73	0.09±0.01	P>F2,t=1.09		71	0.08±0.01	P>F2,t=3.66	***
	F2	173	0.07±0.01	F1>F2,t=1.05		132	0.07±0.01	F1>F2,t=1.08	
	P	13	0.09±0.03	P>F1,t=0.24		13	0.12±0.02	P>F1,t=0.97	
BxB	F1	61	0.08±0.01	P>F2,t=0.51		58	0.09±0.01	P>F2,t=-1.91	
	F2	115	0.07±0.01	F1>F2,t=0.49		116	0.06±0.01	F1>F2,t=1.91	
	P	49	0.11±0.02	P>F1,t=1.40		49	0.07±0.02	P<F1,t=-1.32	
BGxBG	F1	215	0.09±0.01	P>F2,t=1.48		222	0.10±0.01	P<F2,t=-0.89	
	F2	593	0.08±0.01	F1>F2,t=-0.05		615	0.09±0.01	F1>F2,t=1.06	
Inter-population									
	P	42	0.18±0.03	P>F1,t=1.79		42	0.16±0.02	P>F1,t=0.51	
BxO	F1	195	0.14±0.01	P>F2,t=5.41	***	186	0.14±0.01	P>F2,t=4.67	***
	F2	433	0.08±0.01	F1>F2,t=6.44	***	410	0.07±0.01	F1>F2,t=7.50	***
	P	46	0.14±0.02	P>F1,t=1.49		46	0.11±0.02	P>F1,t=0.01	
BGxO	F1	215	0.12±0.01	P>F2,t=2.54		203	0.11±0.01	P>F2,t=1.88	
	F2	444	0.10±0.01	F1>F2,t=1.96		372	0.08±0.01	F1>F2,t=3.17	**
	P	44	0.12±0.02	P<F1,t=-0.33		44	0.16±0.02	P>F1,t=2.22	*
BGxB	F1	208	0.12±0.01	P>F2,t=0.35		215	0.12±0.01	P>F2,t=2.60	**
	F2	582	0.11±0.01	F1>F2,t=1.40		582	0.11±0.01	F1>F2,t=0.74	

O = Oak population, B = Beech population, BG = Botanical garden population. p<0.05 *, p<0.01**, p<0.001***

Table 10b. The FA2 index differences between generations and type of crosses for sternopleural bristles in females (direct and reciprocal crosses); *t test* were used for testing differences in means of SB.

		FA2=mean |R-L| /mean [(R+L)/2]							
		Direct				**Reciprocal**			
cross	generation	N	mean±SE	t test	p	N	mean±SE	t test	p
Intra-population									
	P	16	0.10±0.03	P<F1,t=-0.16		16	0.09±0.03	P<F1,t=-0.52	
OxO	F1	62	0.10±0.01	P>F2,t=0.47		67	0.11±0.01	P>F2,t=1.36	
	F2	199	0.09±0.01	F1>F2,t=1.18		167	0.06±0.01	F1>F2,t=3.69	***
	P	13	0.14±0.06	P>F1,t=1.25		13	0.07±0.04	P<F1,t=-0.61	
BxB	F1	62	0.10±0.01	P>F2,t=2.60	*	55	0.09±0.01	P<F2,t=-0.30	
	F2	144	0.06±0.01	F1>F2,t=2.41	*	138	0.08±0.01	F1>F2,t=0.60	
	P	49	0.08±0.02	P<F1,t=-1.43		49	0.11±0.02	P>F1,t=0.99	
BGxBG	F1	207	0.10±0.01	P<F2,t=-0.71		208	0.10±0.01	P>F2,t=1.21	
	F2	712	0.09±0.01	F1>F2,t=1.58		686	0.09±0.01	F1>F2,t=0.37	
Inter-population									
	P	42	0.11±0.02	P<F1,t=-1.94		42	0.12±0.01	P<F1, t=-1.54	
BxO	F1	192	0.15±0.01	P>F2,t=2.00	*	180	0.15±0.01	P>F2, t=1.91	
	F2	518	0.08±0.01	F1>F2,t=7.88	***	493	0.08±0.01	F1>F2, t=6.39	***
	P	46	0.10±0.01	P<F1,t=-1.27		46	0.13±0.02	P>F1, t=0.58	
BGxO	F1	198	0.12±0.01	P>F2,t=0.25		187	0.12±0.01	P>F2, t=2.63	**
	F2	528	0.10±0.01	F1>F2,t=3.08	**	484	0.09±0.01	F1>F2, t=3.68	***
	P	44	0.16±0.02	P>F1,t=1.01		44	0.13±0.02	P<F1, t=-0.41	
BGxB	F1	202	0.14±0.01	P>F2,t=3.10	**	190	0.14±0.01	P>F2, t=0.61	
	F2	673	0.11±0.01	F1>F2,t=3.58	***	648	0.12±0.01	F1>F2, t=1.97	*

O = Oak population, B = Beech population, BG = Botanical garden population. p<0.05 *, p<0.01**, p<0.001***