

**SYNERGISTIC EFFECT OF ENVIRONMENTAL AND GENOMIC STRESS ON WING
SIZE OF *Drosophila subobscura***

Marija TANASKOVIĆ¹, Zorana KURBALIJA NOVIČIĆ², Bojan KENIG¹, Marija SAVIĆ
VESELINOVIĆ³, Marina STAMENKOVIĆ-RADAK³, Marko ANĐELKOVIĆ⁴

¹University of Belgrade - Institute for Biological Research “Siniša Stanković”, Belgrade, Serbia

²Animal Ecology, Department of Ecology and Genetics, Evolutionary Biology Center, Uppsala
University, Uppsala, Sweden

³Faculty of Biology, University of Belgrade, Belgrade, Serbia

⁴Serbian Academy of Sciences and Arts, Belgrade, Serbia

Tanasković M., Z. Kurbalija Novičić, B. Kenig, M. Savić Veselinović, M. Stamenković-Radak, M. Anđelković (2016): *Synergistic effect of environmental and genomic stress on wing size of Drosophila subobscura*. - Genetika, Vol 48, No.3, 1039 - 1052.

Growing anthropogenic influence on every aspect of environment arise important issues regarding the ability of populations and species to adapt to variant pressures. Lead is one of the most present contaminants in the environment with detrimental influence on organisms and populations. In combination with genomic stress, lead may act synergistically, leading to reduction in adaptive values. We sampled two *Drosophila subobscura* populations, from ecologically different habitats and established differences in genetic backgrounds and population histories. In order to establish different levels of genome heterozygosity, series of intra-line, intra-population and between population crosses were made. The progeny was reared on a standard *Drosophila* medium and a medium with 200µg/mL of lead acetate and right wing of approximately 4000 individuals was used for geometric morphometric analysis of wing size. Results showed that lead significantly reduces wing size and that magnitude of this reduction is dependent on genetic background, indicating synergistic effect of genomic and environmental stress. There is also an indication of strong female origin influence on the outcome of hybridization when source of environmental stress is lead. Our results showed that the genetic structure of populations is of great importance for population

Corresponding author: Marija Tanasković, Department of Genetics of Populations and Ecogenotoxicology, Institute of Biological Research “Siniša Stanković”, University of Belgrade, Despot Stefan Blvd.142, 11060 Belgrade, Serbia; Phone number: +381 11 20 78 328; Fax number: +381 11 27 61 433; email: marija.tanaskovic@ibiss.bg.ac.rs

fitness in anthropogenic induced stressful conditions. Further studies of synergistic effect of genetic and environmental stress are needed, as well as studies of its outcome in natural populations.

Keywords: *Drosophila subobscura*, hybridization, lead pollution, synergistic effect, wing size

INTRODUCTION

Anthropogenic influence does not only change environmental factors in natural habitats but can also lead to habitat fragmentation. Fragmentation of habitat can influence population genetic structure either through increased risk of stochastic fluctuation in population size or changes in distribution pattern of populations (FRANKHAM 2005a; EWERS and DIDHAM, 2006). Also, it could potentially lead to *de novo* population subdivision and a population with challenged genetic structure could be vulnerable to some environmental stressors. Furthermore, genetically diverse and previously isolated populations may come into secondary contact with other populations, and hybridization may occur (WELLENREUTHER *et al.*, 2010). Effect of hybridization depends on fine balance between its positive and negative outcomes, i.e. hybrid vigor and outbreeding depression (LYNCH, 1991; LYNCH and WALSH, 1998; WHITLOCK *et al.*, 2000). Therefore it is not surprising that studies of the effects of different levels of population heterozygosity in many species showed both hybrid vigor and outbreeding depression (TALLMON *et al.*, 2004; KURBALIJA *et al.*, 2010).

Considering growing anthropogenic influence which not only rapidly changes environment, but cause habitat loss and fragmentation which can lead to changes in genetic structure of populations caused by inbreeding and hybridization, it is realistic to expect that growing number of natural population will experience synergistic effect of environmental and genomic stress (FRANKHAM, 2005b). However, literature data regarding synergistic effect of environmental and genomic stress is scarce, and the results obtained are variable. Consequences of combined stresses are both species and stress specific, as well as dependent on the intensity of experienced stress. Also, the consequences depend on the genetic architecture of the studied traits and, in the case of hybridization, on the differences in evolutionary histories of populations coming in contact. When inbreeding is the source of genetic stress, results of synergistic effect is more consistent among taxa and the stresses applied. Therefore, it is clear that inbred individuals are more prone to detrimental effect of environmental stress and that there is linear relationship between inbreeding depression and intensity of stress (FOX and REED, 2011; ENDERS and NUNNEY, 2012). On the other hand, when outbreeding is the source of genetic stress, the outcomes of synergistic effect are more variable. Early studies showed that stressful conditions increases beneficial effect of heterosis (ARMBRUSTER *et al.*, 1997). This may be due the several mechanisms: in stressful conditions masking deleterious effect of recessive alleles is intensified (HOFFMAN and PARSONS, 1991), buffering capacity of heterozygotes is increased (LERNER, 1954) and reduction of deleterious epistatic interactions is more efficient (EDMANDS and DEIMLER, 2004). On the other hand, in the case where there is previous adaptation to stressful environmental conditions, *Chironomus riparius* hybrids obtained from crossing between population adapted to cadmium pollution and population from unpolluted environment rapidly lost cadmium tolerance, suggesting that outbreeding may have detrimental consequence in cases of local adaptation (GROENENDIJK *et al.*, 2002). Also, there is some suggestion that in

interpopulation hybrids fitness response may be environmentally dependent (EDMANDS and DEIMLER, 2004).

Heavy metal pollution is regarded as one of the most important environmental stress factor (PACYNA and PACYNA, 2001) mainly caused by anthropogenic activities, with lead being the most widespread pollutant (NRIAGU and PACYNA, 1988). It has been shown that lead prolongs developmental time (COHN *et al.*, 1992; BERZINS and BUNDY, 2002; JEZIEŃSKA *et al.*, 2009; HUANGA *et al.*, 2014; TANASKOVIC *et al.*, 2015), reduces life span (MASSIE *et al.*, 1992) and even influences complex behavioral traits such as courtship (MASSIE *et al.*, 1992; HIRSCH *et al.*, 2003). Studies show that lead can affect morphological traits, mainly by reducing body size in fish (CANLI and ATLI, 2003), wing size in *Drosophila* (KURBALIJA NOVIĆIĆ *et al.*, 2012), or causing malformation of body structures (VERMEULEN *et al.*, 2000; HAYWOODA *et al.*, 2004; SPARLING *et al.*, 2006).

Drosophila subobscura populations present valuable model in evolutionary research. They are genetically diverse (according to putatively adaptive polymorphism), even on a small geographic scale, with frequently strong and unpredictable consequences of inter-population hybridization (KURBALIJA *et al.*, 2010). There is also an indication of local genetic adaptation to heavy metal pollution and population specific fitness response to heavy metal pollution (KENIG *et al.*, 2013). In recent years, geometric morphometric analysis of *D. subobscura* wings proved to be useful model for genetic and evolution studies and evaluation of number of factors, including genetic and environmental stress (HUEY *et al.*, 2000; GILCHRIST and HUEY, 2004; SANTOS *et al.*, 2004, 2005; KURBALIJA NOVIĆIĆ *et al.*, 2012, PATENKOVIC *et al.*, 2015). Wing development is thoroughly investigated and it is shown that wing size and shape is result of several tightly linked and coordinated processes (de CELIS, 2003; PALSSON and GIBSON, 2004; BAENA-LÓPEZ *et al.*, 2005; DWORKIN *et al.*, 2005; DWORKIN and GIBSON, 2006). Although, wing vein position, and therefore wing size and shape, is highly conserved, numerous studies showed significant additive genetic variance for these traits in *Drosophila* populations. However, amount of genetic variance in total phenotypic variance differs for size and shape. Heritability for wing size is relatively low, suggesting non additive genetic component and rather significant influence of environmental variance and interactions on this trait (BITNER-MATHÉ and KLACZKO, 1999; MORAES *et al.*, 2004; BREUKER *et al.*, 2006). On the other hand, wing shape showed relatively high coefficient of heritability suggesting weaker environmental influence on this component of wing (WEBER *et al.*, 1999, 2001; GILCHRIST and PARTRIDGE, 2001; SANTOS *et al.*, 2004). Considering this, variation of wing size may be more sensitive trait for evaluating effects of environmental and genetic stress.

The present experimental design aims to give a direct empirical evidence that population response on environmental stressor (lead pollution) are significantly dependent on their population structure, demography and evolutionary history and population size. The experimental design modeled the *real life* situation, where we used two distinct natural populations with different evolutionary histories and population structures and also, with different level of experience of lead contamination in their local environments. These two natural populations were experimentally subjected to population subdivision at three different levels (intra-line, intra-population, between populations) in order to test if population origin (population structure, demographic and evolutionary history) and population size (explained indirectly by different levels of heterozygosity in population) significantly influence on population performance in lead contaminated environment by detecting a difference in wing size, which is

functionally important trait and closely related to fitness. Wing size provides a convenient measure of structural body size which is closely related to fitness, it is a complex quantitative trait with low heritability and highly sensitive to the environment (PARTRIDGE *et al.*, 1994).

The specific aim of the study is to evaluate the significance of genetic background (level of heterozygosity) and its ability to moderate a stressful environment (lead pollution) by measuring the variability of wing size as a parameter closely related to fitness.

MATERIALS AND METHODS

For the present study, *Drosophila subobscura* individuals from two ecologically distinct habitats were sampled. First habitat was Botanical garden in Belgrade (44°49' N; 20°28' E), man-made environment in urban part of Belgrade under constant anthropogenic influence. Second habitat was Sicevo Gorge, located in Eastern part of Serbia (43°19' N, 22°08' E), known glacial refugium of the central Balkans with polydominant forests and endemic flora.

In order to establish level of heavy metal pollution, soil analysis of both localities was performed in Institute for Public Health, Belgrade. Heavy metal concentration was determined with Method for assessment of total heavy metal content in soil and sediment according to EPA 200.7 method, routinely performed in this institution.

The flies were sampled using conventional fermented fruit traps and gravid females were individually used for establishing isofemale line (IF). All lines were maintained and all experiments performed under constant laboratory conditions, at 19±0.5°C, approx. 60% relative humidity, light of 300 lux and 12/12 h light/dark cycles.

For the experiment, 32 randomly chosen IF were used for series of lines with different levels of genome heterozygosity. Three main experimental groups by type of cross were established: (i) random crosses within IF line (intra-line IL), (ii) random crosses between IF lines within each of populations (intra-population hybrids IP) and (iii) random crosses between two subsets of IF lines originated from two natural populations (between-population hybrids BP). Additionally, for IP and BP both direct and reciprocal crossings were made in order to detect possible maternal effect. Thus, eight experimental groups by type of cross were established: IL from Botanical Garden and Sicevo Gorge (in further text B and S), direct and reciprocal IP from BG and SG (in further text BB dir, BB rec and SS dir, SS rec) and direct and reciprocal BP (in further text BBS and SSB). All crosses were done with three to five days old virgin flies of both sexes, collected every 24 hours and kept separately. Three males and three females were used for each type of cross (intra-line, intra-population and between-population). The crossing scheme is given in Table 1.

Table 1. The crossing scheme

		Type of cross	
Intra-line (IL)		B	Bi♀xBi♂
		S	Si♀xSi♂
Intra-population (IP)	BB	direct (BB dir)	Bi♀xBj♂
		reciprocal (BB rec)	Bj♀x Bi♂
	SS	direct (SS dir)	Si♀xSj♂
		reciprocal (SS rec)	Sj♀x Si♂
Between-population (BP)	BBxS	direct (BBS)	Bi♀xSi♂
		reciprocal (SSB)	Si♀x Bi♂

After mating and oviposition, 15 eggs from each type of cross were daily transferred to standard *Drosophila* cornmeal medium and medium containing 200 µg/mL lead-acetate (Pb (CH₃COO)₂·3H₂O). In this way, 16 experimental groups (8 on standard medium and 8 on medium with lead) were established. After eclosion, flies were collected, divided according to sex and stored at -20°C for further analysis.

Right wing of each individual was separated from the body and fixed on a microscope slide with double sided tape. Each wing was photographed with a Canon-Leica system, under 40x magnifications. Geometrical morphometric analysis was performed on digital images of wings, resolution 96x96 dpi in different programs of software package Tps, available at <http://life.bio.sunysb.edu/morph>. Between 86 and 176 wings for each sex and group were analyzed.

For wing size analysis 12 distinctive, two-dimensional landmarks was chosen. Landmarks covered the whole wing surface and were placed on the wing vein intersections with other veins and wing margins. Landmarks were recorded on each individual wing in software package tpsDig2 always in same sequence and marked from 1 to 12.

To quantify wing size, centroid size (CS) - the square root of the sum of squared distances of a set of landmarks from their centroid, was computed from the raw coordinates of the landmarks in software package tpsRelw1.44. Normality and homogeneity of data variances were confirmed by Jarque-Bera, Bartlett's and Levene's tests. For analysis of variance in wing size, three-factorial ANOVA with fixed factors treatment, type of cross and sex was used. For further interpretation of statistically significant differences between tested factors, post-hoc Fishers LSD test was used. All statistical analysis was performed in STATISTICA 8.0 software package.

RESULTS

Soil analysis showed higher levels of heavy metals in BG, especially zinc and lead. Concentrations of lead in soil were 68.5 mg/kg in BG and 7.41 mg/kg in SG localities.

Summary of descriptive statistic for wing size of all experimental groups is shown in Table 2a and 2b. From results it can be seen that there is general trend in reduction of wing size in groups exposed to lead contamination. Exception from this trend is BB dir individuals where no change in size was observed.

Three-factorial ANOVA (Table 3) on the variability of the wing size showed that presence of lead in the medium-treatment ($F=416$, $p<0.01$), type of cross ($F=71$, $p<0.01$) and sex ($F=11700$, $p<0.01$) have significant effect on wing size. Also significant *Treatment x Type of cross* ($F=18$, $p<0.01$) and *Type of cross x Sex* interactions was observed ($F=4$, $p<0.01$).

Presence of lead in medium significantly reduces wing size and most profound effect was found in IL groups. Both, females and males from IL groups have smaller wings in the substrate with lead compared to control conditions (Post hoc Fishers LSD $p<0.05$ for all comparisons). Reduction in wing size of IP and BP individuals exposed to lead was observed for the most comparisons (Post hoc Fishers LSD $p<0.05$ for all comparisons). Exception from observed pattern represents BB dir, SS dir and BBS in females and BB dir and BBS in males where no significant difference between flies reared on standard substrate and substrate with lead was observed.

Table 2a. Descriptive statistic for wing size of all experimental groups reared on standard medium

Type of cross	Sex	Standard medium					
		$\bar{X} \pm S.E.$	S.D.	CV	N		
IL	B	female	1248.02 \pm 3.89	42.4	3.4	118	
		male	1136.82 \pm 3.38	39.9	3.5	139	
	S	female	1254.84 \pm 4.19	49.6	4	140	
		male	1134.51 \pm 3.25	39.7	3.5	150	
IP	BB dir	female	1263.84 \pm 3.06	32.9	2.6	116	
		male	1139.34 \pm 3.20	36.3	3.2	128	
	BB rec	female	1277.24 \pm 3.10	35.2	2.8	130	
		male	1149.89 \pm 2.96	35.6	3.1	145	
	SS dir	female	1282.64 \pm 2.39	31.4	2.5	176	
		male	1147.78 \pm 2.59	31	2.7	144	
	SS rec	female	1285.44 \pm 2.78	31.7	2.5	132	
		male	1157.54 \pm 3.23	36.4	3.1	127	
	BP	BBS	female	1283.04 \pm 2.82	32.8	2.6	136
			male	1163.50 \pm 2.41	29.2	2.5	147
SBB		female	1273.25 \pm 2.80	32.8	2.6	137	
		male	1150.65 \pm 2.34	28.8	2.5	151	

\bar{X} - mean value; S.E. - standard error; S.D. - standard deviation; CV - coefficient of variation; N - number of individuals

Table 2b. Descriptive statistic for wing size (centroid size) of all experimental groups reared on medium with lead

Type of cross	Sex	Medium with lead					
		$\bar{X} \pm S.E.$	S.D.	CV	N		
IL	B	female	1230.07 \pm 3.85	42.1	3.4	120	
		male	1109.42 \pm 3.00	32.1	2.9	115	
	S	female	1212.94 \pm 4.52	52.1	4.3	133	
		male	1096.59 \pm 4.59	49.5	4.5	117	
IP	BB dir	female	1263.86 \pm 3.03	35.2	2.8	135	
		male	1141.54 \pm 2.70	31.2	2.7	134	
	BB rec	female	1243.73 \pm 3.06	36.1	2.9	139	
		male	1116.41 \pm 2.70	29.7	2.7	121	
	Ss dir	female	1265.11 \pm 4.38	40.4	3.2	86	
		male	1128.24 \pm 2.76	30	2.7	120	
	Ss rec	female	1250.71 \pm 3.80	39.3	3.1	108	
		male	1119.03 \pm 3.42	33.3	3	96	
	BP	BBS	female	1272.52 \pm 3.10	34.1	2.7	121
			male	1149.66 \pm 3.26	33.4	2.9	108
SBB		female	1247.96 \pm 3.36	38.7	3.1	133	
		male	1125.74 \pm 2.90	33.2	2.9	133	

\bar{X} - mean value; S.E. - standard error; S.

Table 3. Results of a two-way ANOVA for wing size in all experimental groups

Source of variation	df	MS	F	p
Treatment	1	5.54E+05	416	0.000*
Type of cross	7	9.49E+04	71	0.000*
Sex	1	1.56E+07	11700	0.000*
Treatment x Type of cross	7	2.36E+04	18	0.000*
Treatment x Sex	1	567	0	0.514
Type of cross x Sex	7	5131	4	0.000*
Treatment x Type of cross x Sex	7	545	0	0.897

p<0.05*, p<0.01**, p<0.001***

Groups of IL type of cross showed significantly smaller wings compared with IP and BP groups reared on same type of medium but different pattern between sexes was observed. In control conditions, IL females have significantly smaller wings when compared with all IP and BP (Post hoc Fisher LSD $p<0.01$ for all comparisons) with exception of BB dir. Same pattern of reduction was observed in medium with lead (Post hoc Fisher LSD $p<0.01$ for all comparisons), except in B and BB rec comparison where no statistical significance was detected. Males from IL groups generally have smaller wings compared to IP and BP. In control conditions significant reduction of wing size was detected only between B - SS rec, B - BBS; S - SS rec, S - BBS, S - SBB (Post hoc Fisher LSD $p<0.05$ for all comparisons). However, in medium with lead, S males have significantly smaller wing compared with all IP and BP groups and B males have significantly smaller wings when compared with BB dir, SS rec and BBS (Post hoc Fisher LSD $p<0.05$ for all comparisons). (Figure 1)

Results show that there is no difference between most direct and reciprocal crosses on the same type of substrate. Interesting exception from the observed pattern represent comparisons on medium with lead where individuals from BB dir and BBS have significantly larger wings than those from corresponding reciprocal crosses (Post hoc Fisher LSD $p<0.01$ in both comparisons).

Results showed that there are no significant differences between IP and BP groups reared in the same conditions in general. However, there is exception with BB dir group. Females from this group reared on the standard substrate have significantly smaller wings compared with all other females from IP and BP reared in the same conditions with exception of SSB (Post hoc Fisher LSD $p<0.01$ in all comparisons) while such difference was not observed on substrate with lead. Males from this group have significantly smaller wings compared with SS rec and BBS in control conditions (Post hoc Fisher LSD $p<0.05$ in both comparisons) and SS rec in substrate with lead (Post hoc Fisher LSD $p=0.002$). Also, BBS males reared on substrate with lead have significantly larger wings compared with all other IP and BP groups reared in the same conditions, except BB dir (Post hoc Fisher LSD $p<0.001$ in all comparisons).

The significant interaction between type of cross and treatment was detected. In IL crosses reduction of wing size is greater in S individuals. In IP groups, BB dir individual showed the smallest average wing size but no change in wing size was observed between different types of substrate, while all other groups showed wing size reduction in medium lead with slightly greater effect in SS groups. In BP groups, individuals whose mothers originated from Botanical Garden showed no significant reduction in wing size in presence of lead in substrate, while

individuals whose mothers originated from Sicevo Gorge have significantly smaller wing when lead is present in medium (Figure 2.).

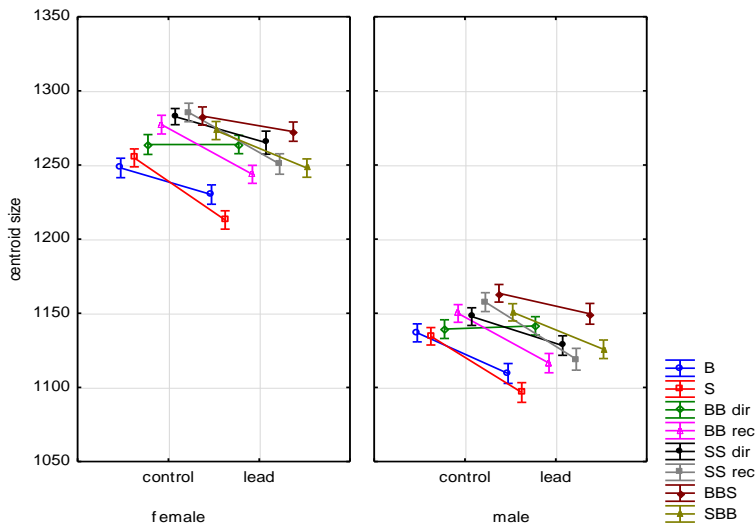


Figure1. Centroid size (in pixels) off all experimental groups divided according to sex in standard substrate and substrate with lead

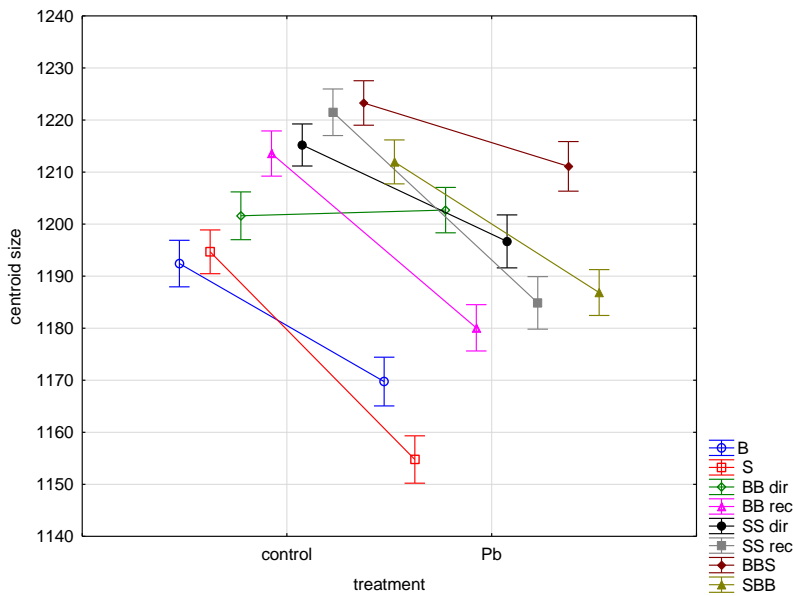


Figure 2. Type of cross*treatment interaction

DISCUSSION

Considering growing, mainly detrimental, anthropogenic influence on every aspect of environment, important issues arise regarding the limits and the ability of populations and species to adapt to different anthropogenic selective pressures. Aimed to investigate changes in wing size of two distinct natural populations under stressful conditions caused by lead pollution, results obtained in this study represent a rare empirical data set using model organism *Drosophila subobscura* with a well-known genetic background and ecology. The populations which differ in genetic backgrounds (STAMENKOVIC-RADAK *et al.*, 2012) each shaped by its specific evolutionary history and microclimatic conditions were sampled from localities characterized by different levels of lead soil contamination. The experimental design was made to detect variation in wing size under stressful environmental conditions among hybrids with different population origin and to estimate if genome hybridization at different levels is able to moderate the effects of this type of stressful environments.

The results showed significant reduction of wing size in flies reared in lead stressed environment compared to control group. Size reduction under stressful lead conditions observed in this study is in concordance with previous results in *D. subobscura* (KURBALIJA NOVICIC *et al.*, 2012; KENIG *et al.*, 2013) suggesting that lead interferes with finely tuned processes of wing development, causing size reduction. It is interesting to note that although developmental time in examined groups is significantly prolonged (TANASKOVIC *et al.*, 2015), under lead stress wing size is reduced, suggesting possible *trade-off* between two traits. Each organism has limited amount of available energy that can be directed in life history processes such as viability, development, reproduction, growth and body size and resource allocation in one trait is usually accompanied by reduction of other trait (VAN NOORDWIJK and DE JONG, 1986; STEARNS, 1992; REZNICK *et al.*, 2000; ROFF, 2002). It is possible that lead exposed individuals needed extended time to complete development, which left them with lesser amount of energy to complete wing development and growth, resulting in reduction of wing size. Following this line of thinking, it is clear that lead not only detrimentally affect fitness components such as developmental time but also fitness proxy, body size.

Results showing significant effect of type of cross leads to two types of conclusion. Foremost, IL individuals had greatest reduction of wing size. These flies are mildly inbred and the most probable cause of these results is expression of deleterious recessive alleles in homozygote state thus leading to reduction of adaptive values and body size (FRANKHAM 2005a; TROTTA *et al.*, 2011). Secondly, there is general trend of increasing in wing size with increased level of whole genome heterozygosity which suggests positive effects of outbreeding. Although, there is no statistically significant difference between IP and BP groups, we may argue that IP heterozygotes have greater buffering capacity to reduce detrimental effects of unfavorable environment and that in BP heterozygotes additional beneficial effects arose from new favorable interaction between loci and/or disruption of negative interaction fixated in original populations (LYNCH, 1991; ERICKSON and FENSTER, 2006; EDMANDS *et al.*, 2009).

The important result of this study is significant interaction between two types of stress which is clear evidence of their synergistic effect. Lead as environmental stressor had most detrimental effect on IP groups leading to highest reduction in wing size. Our results clearly indicate that even relatively small amount of genome homozygotization in stressful environment may lead to significant reduction of adaptive value in tested individuals. Effect of environmental stress is most pronounced in genetically challenged populations, and if there is no standing

genetic variation population won't be able to cope with stressful environment and its adaptive value will drop. This is why human impact on habitat fragmentation is so dangerous from population point of view. With habitat fragmentation, number of individuals that may come in reproductive contact declines, which leads to inbreeding and genome homozygotization and consequently to the reduced genetic variability which is necessary to cope with changing environment. Our results also showed that heterozygous individuals are more resilient to lead stress, further supporting hypothesis that maintaining genetic variation in populations is of uttermost imperative in stressful environments. The results are in concordance with number of research demonstrating that heterosis has higher intensity in stressful environments where masking of detrimental, conditionally expressed recessive alleles is of strong fitness benefit (REED *et al.*, 2002, 2012; JOUBERT and BIJLSMA, 2010; BIJLSMA and LOESCHCKE, 2012).

Finally, the obtained results indicate that evolutionary histories of populations that come in secondary contact have significant effect on outcome of hybridization. We observed significant reduction of wing size in presence of lead only for BP individuals whose mothers originated from unpolluted environment of Sicevo Gorge. This finding indicate that female origin and cytoplasmic influence is of great importance suggesting adaptive significance of mitochondrial gene complexes which together with nuclear DNA controlling incorporated in oxidative phosphorylation electron transport chain (OXPHOS) and energy metabolism of cells. If we assume that mtDNA variation is shaped by natural selection (BALLARD and WHITLOCK, 2004; BALLARD and RAND, 2005; MEIKLEJOHN *et al.*, 2007; CHRISTIE *et al.*, 2011; KAZANCIOGLU and ARNQVIST, 2014), it is possible that females from polluted environments have more efficient OXPHOS enabling their offspring better fitness under stressful conditions.

In conclusion, the results obtained in this study showed that lead reduces wing size and its detrimental effects are more pronounced in individuals that experience low level of inbreeding, indicating synergistic effect of genetic and environmental stress. Results also showed that wing size increase with increased level of genome heterozygosity and that heterozygotes are more stable in stressful environment, favoring hypothesis that positive heterosis could be the prevalent mechanism that stabilizes this trait in a population exposed to lead. There is also an indication that female origin affects wing size suggesting important role of gene complexes of mtDNA in stressful conditions. Finally, the results from our research clearly suggests that that in anthropogenic induced stressful conditions such as lead pollution, the genetic structure of populations is of great importance for population fitness. Therefore, there is a need for further investigation of synergistic effect of genetic and environmental stress and its outcome in natural populations.

ACKNOWLEDGMENTS

Funding for the study was received from the Ministry of Education, Science and Technological Development, Republic of Serbia (Grant No. 173012) for MT, BK, MSR, MA, and from the European Union's Horizon 2020 research and innovation programme under Marie Skłodowska-Curie grant No. 656338 for ZKN.

Received May 31st, 2016

Accepted July 12th, 2016

REFERENCES

- ARMBRUSTER, P., W.E. BRADSHAW, C.M. HOLZAPFEL (1997): Evolution of the genetic architecture underlying fitness in the pitcher-plant mosquito, *Wyeomyia smithii*. *Evolution*, 51: 451-458.

- BAENA-LÓPEZ, L. A., A. BAONZA, A. GARCÍA-BELLIDO (2005): The orientation of cell divisions determines the shape of *Drosophila* organs. *Curr Biol.*, *15*: 1640-1644.
- BALLARD, J.W.O. and D.M. RAND (2005): The population biology of mitochondrial DNA and its phylogenetic implications. *Annu. Rev. Ecol. Evol. Syst.*, *36*: 621-642.
- BALLARD, J.W.O. and M.C. WHITLOCK (2004): The incomplete natural history of mitochondria. *Mol. Ecol.*, *13*: 729-744.
- BERZINS, D.W., K.J. BUNDY (2002): Bioaccumulation of lead in *Xenopus laevis* tadpoles from water and sediment. *Environ. Int.*, *28*: 69-77.
- BIJLSMA, R. and V. LOESCHCKE (2005): Environmental stress, adaptation and evolution: an overview. *J. Evol. Biol.*, *18*: 744-749.
- BIJLSMA, R. and V. LOESCHCKE (2012) Genetic erosion impedes adaptive responses to stressful environments. *Evolutionary Applications*, *5*: 117-129.
- BITNER-MATHÉ B. C. and L.B. KLACZKO (1999): Heritability, phenotypic and genetic correlations of size and shape of *Drosophila mediopunctata* wings. *Heredity*, *83*: 688-696.
- BREUKER, C. J., J.S. PATTERSON, C.P. KLINGENBERG (2006): A single basis for developmental buffering of *Drosophila* wing shape. *PLoS one*, *1*(1), e7.
- CANLI, M. and G. ATLI (2003): The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. *Environ. Pollut.*, *121*: 129-136.
- CHRISTIE, J.S., A. PICORNELL, A. MOYA, M.M. RAMON, J.A. CASTRO (2011): Mitochondrial DNA effects on fitness in *Drosophila subobscura*. *Heredity*, *107*: 239-245.
- COHN, J., D.V. WIDZOWSKI, D.A. CORY-SLECHTA (1992): Lead retards development of *Drosophila melanogaster*. *Comp. Biochem. Physiol.*, *102*:45-9.
- DE CELIS, J.F. (2003): Pattern formation in the *Drosophila* wing: the development of the veins. *Bioessays*, *25*: 443-451.
- DWORKIN, I., A. PALSSON, G. GIBSON (2005): Replication of an Egrf-wing shape association in a wild-caught cohort of *Drosophila melanogaster*. *Genetics*, *169*: 2115-2125.
- DWORKIN, I. and G. GIBSON (2006): Epidermal growth factor receptor and transforming growth factor- β signaling contributes to variation for wing shape in *Drosophila melanogaster*. *Genetics*, *173*: 1417-1431.
- EDMANDS, S. AND J.K. DEIMLER (2004): Local adaptation, intrinsic coadaptation and the effects of environmental stress on interpopulation hybrids in the copepod *Tigriopus californicus*. *J. Exp. Mar. Biol. Ecol.*, *303*: 183-196.
- EDMANDS, S., S.L. NORTHRUP, A.S. HWANG (2009): Maladapted gene complexes within populations of the intertidal copepod *Tigriopus californicus*? *Evolution*, *63*: 2184-2192
- ENDERS, L.S. and L.NUNNEY (2012): Seasonal stress drives predictable changes in inbreeding depression in field-tested captive populations of *Drosophila melanogaster*. *Proceedings of the Royal Society of London B: Biological Sciences*, rspb20121018.
- ERICKSON, D.L. and C.B. FENSTER (2006): Intraspecific hybridization and the recovery of fitness in the native legume *Chamaecrista fasciculata*. *Evolution*, *60*: 225-233
- EWERS, R.M. and R.K. DIDHAM (2006): Continuous response functions for quantifying the strength of edge effects. *J. Appl. Ecol.*, *43*: 527-536.
- FOX, C.W. and D.H. REED (2011): Inbreeding depression increases with environmental stress: an experimental study and meta-analysis. *Evolution*, *65*: 246-258
- FRANKHAM, R. (2005a): Genetics and extinction. *Biological Conservation*, *126*: 131-140.
- FRANKHAM, R. (2005b): Stress and adaptation in conservation genetics. *J. Evol. Biol.*, *18*: 750-755.
- GILCHRIST, A.S. and L. PARTRIDGE (2001): The contrasting genetic architecture of wing size and shape in *Drosophila melanogaster*. *Heredity*, *86*: 144-152.
- GILCHRIST, G.W. and R.B. HUEY (2004): Plastic and genetic variation in wing loading as a function of temperature within and among parallel clines in *Drosophila subobscura*. *Integr. Comp. Biol.*, *44*: 461-470.

- GROENENDIJK, D., S.M.G. LUCKER, M. PLANS, M.H.S. KRAAK, W. ADMIRAAL (2002): Dynamics of metal adaptation in riverine chironomids. *Environ. Pollut.*, *117*: 101-109.
- HAYWOOD, L.K., G.J. ALEXANDER, M.J. BYRNE, E. CUKROWSKA (2004): *Xenopus laevis* embryos and tadpoles as models for testing for pollution by zinc, copper, lead and cadmium. *African Zoology*, *39*: 163-174.
- HIRSCH, H.V.B., J. MERCER, H. SAMBAZIOTIS, M. HUBER, D.T. STARK, T. TORNO-MORLEY, K. HOLLOCHER, H. GHIRADELLA, D.M. RUDEN (2003): Behavioral effects of chronic exposure to low levels of lead in *Drosophila melanogaster*. *Neurotoxicology*, *24*: 435-442.
- HOFFMANN, A.A. and P.A. PARSONS (1991): *Evolutionary Genetics and Environmental Stress*. Oxford University Press, Oxford, UK.
- HUANG, M.Y., R.Y. DUAN, X. JI (2014): Chronic effects of environmentally-relevant concentrations of lead in *Pelophylax nigromaculata* tadpoles: Threshold dose and adverse effects. *Ecotoxicol. Environ. Saf.*, *104*: 310-316.
- HUEY, R.B., G.W. GILCHRIST, M. L. CARLSON, D. BERRIGAN, L. SERRA (2000): Rapid evolution of a geographic cline in size in an introduced fly. *Science*, *287*: 308-309.
- JEZERSKA, B., K. LUGOWSKA, M. WITESKA (2009). The effects of heavy metals on embryonic development of fish (a review). *Fish. Physiol. Biochem.*, *35*: 625-640.
- JOUBERT, D. and R. BIJLSMA (2010): Interplay between habitat fragmentation and climate change: inbreeding affects the response to thermal stress in *Drosophila melanogaster*. *Clim. Res.*, *43*: 57-70.
- KAZANCIOGLU, E. and G. ARNQVIST (2014): The maintenance of mitochondrial genetic variation by negative frequency dependent selection. *Ecol. Lett.*, *17*: 22-27.
- KENIG, B., M. STAMENKOVIC-RADAK, M. ANDELKOVIC (2013): Population specific fitness response of *Drosophila subobscura* to lead pollution. *Insect Sci.*, *20*: 245-253.
- KURBALJA NOVICIC, Z., B., KENIG, J. LUDOSKI, M. STAMENKOVIC-RADAK, M. ANDJELKOVIC (2012): Lead-induced variation in wing size and shape in populations of *Drosophila subobscura*. *Environ. Entomol.*, *41*: 979-988.
- KURBALJA, Z., M. STAMENKOVIC-RADAK, C. PERTOLDI, M. ANDJELKOVIC (2010): Outbreeding causes developmental instability in *Drosophila subobscura*. *Evol. Ecol.*, *24*: 839-864.
- LERNER, I.M. (1954): *Genetic homeostasis*. Edinburgh: Oliver and Boyd.
- LYNCH, M. (1991): The Genetic Interpretation of Inbreeding Depression and Outbreeding Depression. *Evolution*, *45*: 622-629.
- LYNCH, M., B. WALSH (1998): *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Sunderland, Massachusetts.
- MASSIE, H., V. ALELLO, S. WHITNEY (1992): Lead accumulation during aging of *Drosophila* and effect of dietary lead on life span. *Age*, *15*: 47-49
- MEIKLEJOHN, C.D., K.L. MONTTOOTH, D.M. RAND (2007): Positive and negative selection on the mitochondrial genome. *Trends Genet.*, *23*: 259-263.
- MORAES E. M., V.L. SPRESSOLA, P.R.R. PRADO, L.F. COSTA, F.M. SENE (2004): Divergence in wing morphology among sibling species of the *Drosophila buzzatii* cluster. *J. Zool. Syst. Evol. Res.*, *42*: 154-158.
- NRAGU, J.O. and J.M. PACYNA (1988): Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *Nature*, *333*: 134-139.
- PACYNA, J.M. and E.G. PACYNA (2001): An assessment of global and regional emissions of trace metals to the atmosphere from anthropogenic sources worldwide. *Environ. Rev.*, *9*: 269-298.
- PALSSON, A. and G. GIBSON (2000): Quantitative developmental genetic analysis reveals that the ancestral dipteran wing vein prepattern is conserved in *Drosophila melanogaster*. *Dev. Genes Evol.*, *210*: 617-622.
- PARTRIDGE L., B. BARRIE, K. FOWLER, V. FRENCH (1994): Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. *Evolution*, *48*(4), 1269-1276.

- PATENKOVIĆ A., T. SAVIĆ, B. KENIG, Z. KURBALIJA NOVIČIĆ, M. ANDELKOVIĆ (2015): The impact of extremely low frequency electromagnetic field (50 Hz, 0.25 mT) on fitness components and wing traits of *Drosophila subobscura*. *Genetika*, 47: 967-982
- REED, D.H., C.W. FOX, L.S. ENDERS, T.N. KRISTENSEN (2012): Inbreeding-stress interactions: evolutionary and conservation consequences. *Ann. New York Academy of Sci.*, 1256: 33-48.
- REED, D.H., D.A. BRISCOE, R. FRANKHAM (2002): Inbreeding and extinction: The effect of environmental stress and lineage. *Conserv. Genet.*, 3: 301-307.
- REZNICK, D., L. NUNNEY, A. TESSIER (2000): Big houses, big cars, superfleas and the cost of reproduction. *Trends Ecol. Evol.* 15:421-425.
- ROFF D.A. (2002): *Life History Evolution*. Sinauer Associates, Sunderland, MA, USA.
- SANTO,S M., P. F. IRIARTE, W. CÉSPEDES, J. BALANYÀ, A. FONTDEVILA, L. SERRA (2004): Swift laboratory thermal evolution of wing shape (but not size) in *Drosophila subobscura* and its relationship with chromosomal inversion polymorphism. *J. Evol. Biol.*, 17: 841-855.
- SANTO,S M., W. CÉSPEDES, J. BALANYA, V. TROTTA, F. C. CALBOLI, A. FONTDEVILA, L. SERRA (2005): Temperature-related genetic changes in laboratory populations of *Drosophila subobscura*: evidence against simple climatic-based explanations for latitudinal clines. *Am. Nat.*, 165: 258-273.
- SPARLING, D. W., S. KREST, M. ORTIZ-SANTALIESTRA (2006): Effects of lead-contaminated sediment on *Rana sphenocephala* tadpoles. *Arch. Environ. Contam. Toxicol.*, 51: 458-466.
- STAMENKOVIC-RADAK, M., M. JELIC, Z. NOVICIC KURBALIJA, B. KENIG, M. TANASKOVIC, M. ANDJELKOVIC (2012): Balkan glacial history and modern *Drosophila subobscura* population genetics. *Evol. Ecol. Res.*, 14: 839-858.
- STEARNS, S.C. (1992): *The Evolution of Life Histories*. Oxford University Press, Oxford.
- TALLMON, D.A., G. LUIKART, R.S. WAPLES (2004): The alluring simplicity and complex reality of genetic rescue. *Trends Ecol. Evol.*, 19: 489-496.
- TANASKOVIC, M., Z. KURBALIJA NOVICIC, B. KENIG, M. STAMENKOVIC-RADAK, M. ANDJELKOVIC (2015): Effect of lead pollution on fitness and its dependence on heterozygosity in *Drosophila subobscura*. *J Genet.*, 94(4), 643-649.
- TROTTA, V., S. CAVICCHI, D. GUERRA, D.H. ANDERSEN, G.A. BABBITT, T.N. KRISTENSEN, K.S. PEDERSEN, V. LOESCHCKE, C. PERTOLDI (2011), Allometric and non-allometric consequences of inbreeding on *Drosophila melanogaster* wings. *Biol. J. Linn. Soc.*, 102: 626-634
- VAN NOORDWIJK, A.J. and G. DE JONG (1986): Acquisition and allocation of resources: their influence on variation in life history tactics. *Am. Nat.*, 128: 137-142.
- VERMEULEN, A.C., G. LIBERLOO, P. DUMONT, F. OLLEVIER, B. GODDEERIS (2000): Exposure of *Chironomus riparius* larvae (diptera) to lead, mercury and β -sitosterol: effects on mouthpart deformation and moulting. *Chemosphere*, 41: 1581-1591.
- WEBER, K., R. EISMAN, L. MOREY, A. PATTY, J. SPARKS, M. TAUSEK, Z.B. ZENG (1999): An analysis of polygenes affecting wing shape on chromosome 3 in *Drosophila melanogaster*. *Genetics*, 153: 773-786.
- WEBER, K., R. EISMAN, S. HIGGINS, L. MOREY, A. PATTY, M. TAUSEK, Z.B. ZENG (2001): An analysis of polygenes affecting wing shape on chromosome 2 in *Drosophila melanogaster*. *Genetics*, 159: 1045-1057.
- WELLENREUTHER, M., E. VERCKEN, E.I. SVENSSON (2010): A role for ecology in male mate discrimination of immigrant females in *Calopteryx damselflies*? *Biol. J. Linn. Soc.*, 100: 506-518.
- WHITLOCK, M.C., P.K. INGVARSSON, T. HATFIELD (2000): Local drift load and the heterosis of interconnected populations. *Heredity*, 84: 452-457

**SINERGISTIČKI EFEKAT SREDINSKOG I GENOMSKOG STRESA NA VELIČINU
KRILA KOD JEDINKI *Drosophila subobscura***

Marija TANASKOVIĆ¹, Zorana KURBALIJA NOVIČIĆ², Bojan KENIG¹, Marija SAVIĆ
VESELINOVIĆ³, Marina STAMENKOVIĆ-RADAK³, Marko ANĐELKOVIĆ⁴

¹Univerzitet u Beogradu, Institut za biološka istraživanja "Siniša Stanković", Beograd, Srbija

²Animal Ecology, Department of Ecology and Genetics, Evolutionary Biology Center, Uppsala
University, Uppsala, Sweden

³Univerzitet u Beogradu, Biološki fakultet, Beograd, Srbija

⁴Srpska akademija nauka i umetnosti, Beograd, Srbija

Izvod

Rastući antropogeni uticaj na sve aspekte životne sredine nameće važna pitanja koja se tiču granica i sposobnosti prirodnih populacija i vrsta da odgovore na simultane selektivne pritiske izazvane ovim uticajem. Olovo predstavlja jedan od najrasprostranjenijih zagađivača sa dokazanim štetnim efektima na organizme i populacije. U kombinaciji sa genomskim stresom, olovo može delovati sinergistički, što posledično može dovesti do pada adaptivne vrednosti. Za ovu studiju uzorkovane su jedinke *Drosophila subobscura*, poreklom sa ekološki različitih staništa za koje je ranije utvrđeno da se razlikuju po populacionoj strukturi i evolucionim istotijama. U cilju uspostavljanja različitih nivoa heterozigotnosti genoma, napravljene su serije unutar-linijskih, unutar-populacionih i među-populacionih ukrštanja. Potomstvo iz ovih ukrštanja uzgajano je na standardnom *Drosophila* supstratu i supstratu sa 200µg/mL olovo acetata i desno krilo oko 4000 jedinki je iskorišćeno za geometrijsko morfometrijsku analizu veličine krila. Rezultati pokazuju da olovo značajno smanjuje veličinu krila i da stепен redukcije zavisi genetičke pozadine, ukazujući na sinergistički efekat genomskog i sredinskog stresa. Takođe, postoje i indikacije da poreklo ženki ima veliki uticaj na ishod hibridizacije u prisustvu olova. Naši rezultati pokazuju da je genetička struktura populacije od velike važnosti za adaptivnu vrednost populacija u slučajevima antropogeno izazvanog sredinskog stresa.

Primljeno 31. V. 2016.

Odobreno 12. VII. 2016.