

**RELATIONSHIP BETWEEN CHROMOSOMAL AND MITOCHONDRIAL
DNA VARIABILITY OF *Drosophila subobscura* POPULATION FROM THE
LAZAR'S RIVER CANYON**

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The genetic structure of *Drosophila subobscura* population from the
Lazar's River Canyon (Serbia) was studied with respect to restriction site
polymorphism of mitochondrial DNA and chromosomal inversion
polymorphism. The aim was to shed more light on the role of cytonuclear
interactions in shaping mitochondrial DNA variability in this species.
Similar to other populations of *D. subobscura* two main haplotypes (I and

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II) were found, as well as less common ones that appeared at very low frequencies. The frequency distribution of haplotypes did not depart from neutrality. We did not find statistically significant linkage disequilibrium between the haplotypes belonging to haplogroups I and II and any of the chromosomal arrangements. However, when we compared the data in hereby analyzed population and two previously analyzed populations we observed that haplotype I is more frequent in populations where standard inversion arrangements are less frequent. Pattern of the observed mitochondrial variability could be influenced either directly by environmental variability or through environmentally specific cytonuclear coadaptation.

Key words: *Drosophila subobscura*, cytonuclear coadaptation, inversion polymorphism, mtDNA

INTRODUCTION

Drosophila subobscura is a Palearctic species distributed from North Africa to Scandinavia and from some Atlantic islands to the Middle East. Few decades ago it invaded South and North America where it quickly broaden its range. The population genetics of *D. subobscura* has been extensively studied with respect to several nuclear genetic markers: allozymes (CASTRO *et al.*, 1999), chromosomal inversion polymorphism (IP) (JELIĆ *et al.*, 2009; KENIG *et al.*, 2010), microsatellites (PASCUAL *et al.*, 2001; KURBALIJA NOVIČIĆ *et al.*, 2011); as also with mitochondrial DNA (mtDNA) markers (OLIVER *et al.*, 2002; BREHM *et al.*, 2004).

In reference to mtDNA variation, the results from the Restriction Site Analysis (RSA) of the whole mtDNA genome show genetic homogeneity throughout the species range with the prevalence of two, almost equally frequent haplotypes (named I and II) as well as less common ones that appear at low frequencies (CASTRO *et al.*, 1999; CHRISTIE *et al.*, 2010; JELIĆ *et al.*, 2012). Highly consistent pattern of variability to RSA is observed if nucleotide diversity of mtDNA *ND5* gene is analyzed (CASTRO *et al.*, 2010).

Studies conducted so far largely identified the evolutionary forces responsible for the observed distribution of mtDNA haplotypes in natural populations of this species. Among them genetic drift is consider as an important factor which probably acted through bottlenecks induced by glaciations, and is still acting through seasonal populations decays induced by pesimal conditions during cold winters and dry summers (CHRISTIE *et al.*, 2010; CASTRO *et al.*, 2010). However, the two main haplotypes show adaptive differences in the laboratory which are probably dependable on the nuclear genetic background. While on their own nuclear backgrounds they show adaptive differences in some life history traits and mating behavior (CHRISTIE *et al.*, 2004; CASTRO *et al.*, 2003), the differences are not observed when the nuclear background is uniformed (CHRISTIE *et al.*, 2011). This strongly suggests the role of cytonuclear coadaptation, rather than the direct action of natural selection in shaping the variability of mtDNA. *Drosophila subobscura* offers special opportunity for detecting the action of cytonuclear coadaptation in shaping

mtDNA variability since it is rich in inversion polymorphisms (KRIMBAS, 1993), which has adaptive value (PEGUEROLAS *et al.*, 2010). Additionally, different inversion arrangements with overlapping chromosomal regions inside inversion differ in their allelic content (HOFFMANN *et al.*, 2004). This leads to the conclusion that if there is coadaptation between different mtDNA haplotypes and different alleles harbored in different inversions than the non-random association of the two genetic markers is expected. Previous studies have dealt with the associations of mtDNA haplotypes and IP in natural populations of this species. OLIVER *et al.*, (2002) observed linkage disequilibrium (LD) between the two most frequent haplotypes and the arrangements of the J chromosome in a population from the Island of Majorca (Spain). This was not the case in the study of two Balkan populations (JELIĆ *et al.*, 2012). This population specific pattern of LD could be attributed to temporal or spatial differences in the action of ecologically specific selective pressures, geographical setting as well as to historical processes (JELIĆ *et al.*, 2012).

The aim of this paper was to complement the data on LD between mtDNA and IP in populations of *D. subobscura* with the analysis of another natural population from the Balkan Peninsula. We also discuss the pattern of relationship between the two genetic markers using data on LD from previously tested populations

MATERIALS AND METHODS

D. subobscura flies were collected in the Lazar's River Canyon (44°1'42.17"N, 21°57'28.80"E) in Eastern Serbia in June 2011 using nets and conventional fruit traps. Isofemale strains (IF) were derived, each from a gravid female collected in the wild. Thirty eight IF were formed and then maintained under constant laboratory conditions, at 19°C, ~60% relative humidity, light of 300 lux, and a photoperiod of 12 h light: 12 h dark. When the F₁ larvae appeared the progeny of IF strains were used to determine maternal mitochondrial haplotypes, while F₁ males were karyotyped for chromosomal arrangements. Experimental protocol for mtDNA extraction, digestion with five restriction enzymes, haplotype designation and inversion polymorphism analysis were the same as in JELIĆ *et al.*, (2012). Additionally, nine males captured in the wild were karyotyped, and since it was not possible to obtain enough DNA for digestion with five enzymes, their mtDNA *ND5* fragment was amplified according to GARCÍA-MARTÍNEZ *et al.*, (1998), and digested with *Hae*III restriction enzyme to determine whether it belongs to haplotype I and its derivatives (A pattern of restriction), or haplotype II and its derivatives (C pattern of restriction). In order to exclude incompatibility promoted by the presence of *Wolbachia* a PCR assay was conducted according to GARCÍA-MARTÍNEZ *et al.*, (1998) using positive controls as explained in JELIĆ *et al.*, (2012).

To test departure from neutrality for the mtDNA haplotype distribution in the analyzed population the Tajima's D-test was used (TAJIMA, 1989). The procedure of testing linkage disequilibrium was the same as in OLIVER *et al.*, (2002), and JELIĆ *et al.*, (2012) and included the calculations of D and D' values, followed by the

Fisher's exact test for independence in 2 x 2 tables. Considering small number of captured females and impossibility of exact determination of haplotypes in additional males, rare haplotypes determined in females, and imprecisely designated haplotypes in males were grouped to haplogroup I (haplotype I and haplotypes derived from it) and haplogroup II (haplotype II and haplotypes derived from it).

RESULTS AND DISCUSSION

All IF strains were negative for the presence of *Wolbachia*. Additionally, other signs of cytoplasmatic incompatibility in crosses with different strains were not detected.

The pattern of mtDNA variability in the analyzed population is similar to that found in other populations (CHRISTIE *et al.*, 2010; JELIĆ *et al.*, 2012). However, contrary to other populations from the Northern Balkan Peninsula haplotype I showed higher frequency than haplotype II (Table 1.). Climatic factors may have influence on the haplotype distribution. CHRISTIE *et al.*, (2004) showed that in laboratory conditions survival of haplotype II at optimal density was higher than that of haplotype I. They hypothesized that this advantage of haplotype II persists in nature when seasonal conditions are extreme. This is in agreement with the seasonal changes of haplotype distribution. Haplotype I reaches its peak in June, while the frequency of haplotype II rises in the autumn (CHRISTIE *et al.*, 2010).

Table 1. Percentage of isofemale strains (IF) with different haplotypes and restriction patterns of those haplotypes in *Drosophila subobscura* from the Lazar's River Canyon.

Haplotype	Percentage (%) of IF	Restriction pattern				
		<i>EcoRI</i>	<i>EcoRV</i>	<i>HaeIII</i>	<i>HindIII</i>	<i>HpaII</i>
I	52,63	A	A	A	A	A
II	42,11	A	A	C	A	A
IX	2,63	A	A	A	A	F
X	2,63	A	A	C	A	C

Note: Out of 9 additionally analyzed males two belong to haplogroup I and seven to haplogroup II

The Tajima D-test was used to detect departures of the mtDNA haplotype distribution from neutrality. The observed D value was -0,3121, and was not significantly different from zero, indicating that the variability of mtDNA haplotypes did not significantly depart from neutrality.

The chromosomal arrangement frequencies within haplogroups are presented in Table 2. Statistical tests demonstrate absence of linkage disequilibria between chromosomal arrangements and mtDNA haplogroups (Table 3.). This result is in agreement to two other populations from the Balkan Peninsula.

Table 2. Chromosomal arrangement frequencies within haplogroup I and haplogroup II in *Drosophila subobscura* population from the Lazar's River Canyon

Chromosomal arrangement	Haplogroup I	Haplogroup II	Diff.
A _{ST}	0,5652	0,4167	0,1486
A ₁	0,3913	0,4167	0,0254
A ₂	0,0435	0,1667	0,1232
J _{ST}	0,1739	0,1875	0,0136
J ₁	0,8261	0,8125	0,0136
U _{ST}	0,0870	0,0000	0,0870
U ₁₊₂	0,5435	0,5833	0,0399
U ₁₊₂₊₆	0,3261	0,3958	0,0697
U ₁₊₂₊₇	0,0435	0,0208	0,0226
E _{ST}	0,1739	0,1458	0,0281
E ₈	0,2174	0,2917	0,0743
E ₁₊₂	0,1087	0,0417	0,0670
E ₁₊₂₊₉	0,5000	0,5208	0,0208
O _{ST}	0,2174	0,1250	0,0924
O ₆	0,0217	0,0000	0,0217
O ₃₊₄	0,5000	0,6042	0,1042
O ₃₊₄₊₁	0,1304	0,1250	0,0054
O ₃₊₄₊₂	0,0435	0,0208	0,0226
O ₃₊₄₊₇	0,0435	0,0208	0,0226
O ₃₊₄₊₂₂	0,0435	0,1042	0,0607

Note: Diff, absolute difference between haplogroup frequencies with larger values, suggesting stronger associations with specific chromosomal arrangements.

Here we make an interesting observation on the frequency of mtDNA haplotype I and IP in the analyzed population and two other populations from the Balkan Peninsula (JELIĆ *et al.*, 2012). The lowest frequency of haplotype I is observed in population from the Derventa River Gorge (25,8%), followed by population from Sićevo Gorge (32,4%), and finally in here-by analyzed population

(52,6%). This is mirrored by the same order of decrease in frequency of standard chromosomal arrangements, which are considered as cold adapted (MENOZZI and KRIMBAS, 1992), and hence increase in frequency of chromosomes with warm adapted inversions. There is only exception for the frequency of standard gene arrangement in the U and O chromosomes in the population from the Lazar's River Canyon, which are not the lowest among three populations (Table 4.). Therefore, it appears that haplotype I follows the pattern of chromosomes with inversions. This is consistent with the observation that in the warmest period of the year haplotype I prevails over haplotype II (CHRISTIE *et al.*, 2010). There are two phenomena that could explain such observation. Firstly, the increase in frequency of haplotype I with the increase in frequencies of chromosomes with inversions is independently resulting from environmental variability. On the other hand there could be a complex epistasis between mtDNA and loci on several chromosomes, dependable upon environmental conditions that could not be detected if we observe interaction between singular chromosomes and mtDNA haplotypes. And indeed, environmentally specific epistasis is observed between mtDNA and nuclear genes in insect species (ARNQUIST *et al.*, 2010).

Table 3. Values of D and D' between chromosomal arrangements and haplogroup I in *Drosophila subobscura* population from the Lazar's River Canyon

Chromosomal arrangement	D	D'	p	p_{corr}
A _{ST}	0,0371	0,1486	0,7569	1,0000
A ₁	-0,0063	-0,0320	0,7569	1,0000
J _{ST}	-0,0034	-0,0384	1,0000	1,0000
J ₁	0,0034	0,0384	1,0000	1,0000
U ₁₊₂	-0,0100	-0,0447	0,8338	1,0000
U ₁₊₂₊₆	-0,0174	-0,0985	0,6650	1,0000
E _{ST}	0,0070	0,0861	0,7771	1,0000
E ₈	-0,0186	-0,1486	0,6330	1,0000
E ₁₊₂₊₉	-0,0052	-0,0208	1,0000	1,0000
O _{ST}	0,0231	0,2656	0,2753	1,0000
O ₃₊₄	-0,0260	-0,1141	0,8466	1,0000
O ₃₊₄₊₁	0,0014	0,0208	1,0000	1,0000

Note: The results for haplogroup II are the same as for haplogroup I but with sign change. p_{corr} , probability corrected by the sequential Bonferroni test.

Table 4. Frequencies of standard gene arrangements in *Drosophila subobscura* populations from the Derventa River Gorge (T), Sićevo Gorge (S) and Lazar's River Canyon (L)

Chromosomal arrangement	Populations		
	T	S	L
A _{ST}	0,6452	0,5605	0,4894
J _{ST}	0,3549	0,2433	0,1808
U _{ST}	0,0807	0,0135	0,0426
E _{ST}	0,3387	0,2702	0,1596
O _{ST}	0,2177	0,1217	0,1702

Note: Gene arrangement frequencies for populations T and S are calculated based on the data from JELIC *et al.*, (2012).

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REFERENCES

- ARNQUIST, G., D.K. DOWLING, P. EADY, L. GAY, T. TRAGENZA, M. TUDA and D.J. HOSKEN (2010): Genetic architecture of metabolic rate: Environmental specific epistasis between mitochondrial and nuclear genes in an insect. *Evolution*, *64* (12): 3354-3363.
- BREHM, A., D.J. HARRIS, M. HERNÁNDEZ, J.A. PEREZ, J.M. LARRUGA, F.M. PINTO and A.M. GONZÁLEZ (2004): Phylogeography of *Drosophila subobscura* from north Atlantic islands inferred from mtDNA A + T rich region sequences. *Mol. Phylogenet. Evol.* *30* (3): 829-834.
- CASTRO, J.A., M. RAMON, A. PICORNELL and A. MOYA (1999): The genetic structure of *Drosophila subobscura* populations from the islands of Majorca and Minorca (Balearic Islands, Spain) based on allozymes and mitochondrial DNA. *Heredity*, *83* (3): 271-279.
- CASTRO, J.A., P. OLIVER, J.S. CHRISTIE, A. PICORNELL, M. RAMON and A. MOYA (2003): Assortative mating and fertility in two *Drosophila subobscura* strains with different mitochondrial DNA haplotypes. *Genetica*, *119* (3): 295-301.
- CASTRO, J.A., E. BARRIO, A. GONZÁLEZ, A. PICORNELL, M.M. RAMON and A. MOYA (2010): Nucleotide diversity of a ND5 fragment confirms that population expansion is the most suitable explanation for the mtDNA haplotype polymorphism of *Drosophila subobscura*. *Genetica*, *138* (8): 819-829.
- CHRISTIE, J.S., J.A. CASTRO, P. OLIVER, A. PICORNELL, M.M. RAMON and A. MOYA (2004): Fitness and life-history traits of the two major mitochondrial DNA haplotypes of *Drosophila subobscura*. *Heredity*, *93* (4): 371-378.

- CHRISTIE, J.S., A. PICORNELL, A. MOYA, M.M. RAMON and J.A. CASTRO (2010): Dynamics of the mtDNA haplotype variability in a *Drosophila subobscura* population over a two-year period. *Open Evol. J.*, 4 (1): 23-30.
- CHRISTIE, J.S., A. PICORNELL, A. MOYA, M.M. RAMON and J.A. CASTRO (2011): Mitochondrial DNA effects on fitness in *Drosophila subobscura*. *Heredity*, 107 (3): 239–245.
- GARCÍA-MARTÍNEZ, J., J.A. CASTRO, M. RAMÓN, A. LATORRE and A. MOYA (1998): Mitochondrial DNA haplotype frequency in natural and experimental populations of *Drosophila subobscura*. *Genetics*, 149 (3): 1377-1382.
- HOFFMANN, A.A., C.M. SGRÒ and A.R. WEEKS (2004): Chromosomal inversion polymorphism and adaptation. *Trends. Ecol. Evol.*, 19 (9): 482-488.
- JELIĆ, M., B. KENIG, Z. KURBALIJA, M. STAMENKOVIĆ-RADAK and M. ANDELKOVIĆ (2009): Intra-species differentiation among *Drosophila subobscura* from different habitats in Serbia. *Arch. Biol. Sci. Belgrade*, 61 (3): 513-521.
- JELIĆ, M., J.A. CASTRO, Z. KURBALIJA NOVIČIĆ, B. KENIG, D. DIMITRIJEVIĆ, M. SAVIĆ VESELINOVIĆ, M. JOVANOVIĆ, D. MILOVANOVIĆ, M. STAMENKOVIĆ-RADAK and M. ANDELKOVIĆ (2012): Absence of linkage disequilibria between chromosomal arrangements and mtDNA haplotypes in natural populations of *Drosophila subobscura* from the Balkan Peninsula. *Genome*, 55 (3): 214-221.
- KENIG, B., M. JELIĆ, Z. KURBALIJA, M. STAMENKOVIĆ-RADAK and M. ANDELKOVIĆ (2010): Inversion polymorphism in populations of *Drosophila subobscura* from urban and non-urban environments. *Arch. Biol. Sci. Belgrade*, 62 (3): 565-574.
- KRIMBAS, C.B. (1993): *Drosophila subobscura*: biology, genetics, and inversion polymorphism. Verlag Dr. Kovac, Hamburg.
- KURBALIJA-NOVIČIĆ, Z., M. JELIĆ, M. JOVANOVIĆ, D. DIMITRIJEVIĆ, M. SAVIĆ VESELINOVIĆ, M. STAMENKOVIĆ-RADAK and M. ANDELKOVIĆ (2011): Microsatellite variability of *Drosophila subobscura* populations from central Balkans. *Evol. Ecol. Res.*, 13 (5): 479-494.
- MENOZZI, P. and C.B. KRIMBAS (1992): The inversion polymorphism of *Drosophila subobscura* revisited: synthetic maps of gene arrangement frequencies and their interpretation. *J. Evol. Biol.*, 5 (4): 625-641.
- OLIVER, P., J.A. CASTRO, A. PICORNELL, M.M. RAMON, E. SOLÉ, J. BALANYÀ, L. SERRA, A. LATORRE and A. MOYA (2002): Linkage disequilibria between mtDNA haplotypes and chromosomal arrangements in a natural population of *Drosophila subobscura*. *Heredity*, 89 (2): 133-138.
- PASCUAL, M., C.F. AQUADRO, V. SOTO and L. SERRA (2001): Microsatellite variation in colonizing and Palearctic populations of *Drosophila subobscura*. *Mol. Biol. Evol.*, 18 (5): 731-740.
- PEGUEROLES, C., V. ORDÓÑEZ, F. MESTRES and M. PASCUAL (2010): Recombination and selection in the maintenance of the adaptive value of inversions. *J. Evol. Biol.*, 23 (12): 2709-2717.
- TAJIMA, F. (1989): Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, 123 (3): 585-595.

**ANALIZA POVEZANOSTI HROMOZOMSKE I MITOHONDRIJALNE
DNK VARIJABILNOSTI VRSTE *Drosophila subobscura*
IZ KANJONA LAZAREVE REKE**

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U ovom radu analizirana je genetička varijabilnost restrikcionih mesta mitohondrijalne DNK, kao i varijabilnost inverzionog polimorfizma u populaciji *Drosophila subobscura* sakupljenoj u kanjonu Lazareve reke. Cilj je bio rasvetljavanje uloge citonuklearnih interakcija u oblikovanju varijabilnosti mitohondrijalne DNK. Utvrđeno je prisustvo dva dominantna haplotipa (I i II) kao i dva haplotipa niske učestalosti. Nije utvrđena statistički značajna neravnoteža vezanosti između haplotipova grupisanih u haplogrupe I i II i nijednog od hromozomskih aranžmana. Međutim, upoređivanjem podataka prethodno analiziranih populacija i populacije analizirane u ovom radu, primećuje se povećanje učestalosti haplotipa I sa smanjenjem učestalosti standardnih hromozomskih aranžmana. Ovakav obrazac ukazuje ili na direktan uticaj sredinskih činilaca na varijabilnost mitohondrijalne DNK ili na sredinski zavisnu citonuklearnu koadaptaciju.

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