

INVERSION POLYMORPHISM IN POPULATIONS OF *DROSOPHILA SUBOBSCURA* FROM URBAN AND NON-URBAN ENVIRONMENTS

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Abstract – Populations of *Drosophila subobscura* from the urban area of Belgrade and from the locality, Deliblato, which is not under strong anthropogenic influence, were studied with the aim to characterize and compare their genetic structure by examining chromosomal inversion polymorphism. Additional analysis and comparison of this type of polymorphism with several other populations from different habitats in the central Balkans, was done. The obtained results indicate higher heterozygosity in the population from Belgrade. Despite being ecologically marginal and under strong and complex influences, this population did not show a decline in the number of inversions and it is not highly differentiated compared to the referent populations.

Keywords: *Drosophila subobscura*, inversion polymorphism, urban environment, clinal variation

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INTRODUCTION

Urban areas are man-made ecosystems with extreme life conditions, due to the varying climate, poor availability of native trophic resources and the presence of new competitors. Another ecological consequence of urban expansion is pollution, which significantly alters the environment by impairing the quality of air, soil, water and natural resources (Marcus and Detwyler, 1972; Rebele, 1994). On the other hand, urban environments could potentially offer new substrates to be exploited as well as new evolutionary opportunities. Despite being conspicuous and influential features of the biosphere, urban ecosystems have been neglected in ecological research (McIntyre et al., 2001). There are relatively few general studies on arthropods in urban environments (McIntyre, 2000). The species of the family Drosophilidae are excellent biological models for studies of the response of organisms to urbanization because they are abundant in both urban and non-urban settings and there is plenty biological information on these species (Parsons, 1991; Powell, 1997; Avondet et al., 2003). In certain

Drosophila species, chromosomal inversion polymorphism is higher in urban populations than rural populations. It has been suggested that the high degree of urbanization leads to the increase of ecological niches and consequently to higher chromosomal variability (Singh, 1994; Valiati and Valente, 1997). Valente et al. (1993; 2003) showed that changes in *D. willistoni* distribution reflect a response of chromosomal inversion frequency to ecological parameters along an urbanization gradient in the city of Porto Alegre (Brazil).

Drosophila subobscura is a typical Palearctic species found throughout Europe, in the Middle East, northern Africa, and it has recently colonized the New World. In this species, a very rich inversion polymorphism has been observed at all five acrocentric chromosomes of the set. More than 60 different inversions forming more than 90 different chromosomal gene arrangements have been described (for a review see Krimbas, 1992; 1993). Many of the chromosomal gene arrangements exhibit an inter-population variability correlated with latitude (Prevosti, 1966; Prevosti et al., 1984, 1988; Balanya

et al., 2003), but some of them are rare and/or restricted to limited areas. The standard arrangements are more frequent in northern populations and are followed by a gradual decrease in frequency towards the south. However, single or complex inversion arrangements show the opposite trend (Sole et al., 2002). Although historical processes cannot be ruled out, selection by climatic variables seems to have shaped the overall pattern of geographic frequency distribution of the various gene arrangements (Menozzi and Krimbas, 1992). It is generally accepted that the latitudinal clines of chromosomal polymorphism are due to environmental factors among which temperature probably has the most important role (Santos et al. 2005). Shortly after the colonization of North and South America by *D. subobscura*, chromosome inversions evolved latitudinal frequency clines in the same direction as in Europe (Ayala et al., 1989), which suggests that the inversion polymorphism of *D. subobscura* is adaptive (Prevosti et al., 1988).

Taking into account that this type of polymorphism is clearly responding to various environmental conditions, it could be a suitable parameter for the detection of population differentiation due to adaptation to distinctive environmental conditions.

The main objective of the present study was to examine the structure of inversion polymorphism of two *D. subobscura* populations. One is from the Botanical Garden in Belgrade, a locality that represents the urban environment and which is under strong and complex anthropogenic influence, and the other population is from Deliblato, a locality relatively distant from any urban environment and considered to be unpolluted (Kadović and Knežević, 2002). It is known that *D. subobscura* populations inhabit places disturbed by man (Goni et al., 1998), but this kind of environment is regarded as unpredictable and populations in urban environments are considered as ecologically marginal. We compared inversion polymorphism parameters obtained for these two populations with the ones expected from their geographical position within species distribution.

MATERIAL AND METHODS

Drosophila subobscura flies were collected using fermented fruit traps from Botanical Garden in Belgrade (44°49' N; 20°28' E) and Deliblato (N 44°49'; 21°07' E).

Analysis of inversion polymorphism was carried out with males caught from each population. The males were individually crossed with virgin females from the Künsnacht laboratory line, which is homozygous for standard gene arrangement at all chromosomes. The salivary glands from third-instar larvae were squashed and their chromosomes stained with aceto-orcein solution. Eight larvae were analyzed from the progeny of each cross. Gene arrangements were identified using the chromosome map of Kunzhe-Mühl and Müller (1958), the gene arrangement designation of Kunzhe-Mühl and Sperlich (1955). Thirty one male (62 autosomes and 31 sex chromosomes) from the Belgrade population and 33 males (66 autosomes and 33 sex chromosomes) from the Deliblato population, were analyzed.

Z-statistics (Zar, 1999) was used to assess the differences between the frequencies of gene arrangements individually in the two studied populations. The G-test (Sokal and Rohlf, 1980) was performed to determine the heterogeneity of gene arrangement frequencies between the populations studied at all five chromosomes separately and for all chromosomes in total.

Sequential Bonfferoni correction was applied to adjust for multiple pairwise comparisons (Rice, 1989).

The inversion polymorphism parameters, index of free recombination (IFR) and index of heterozygosity (HZ), were derived from the arrangement frequencies according to the description of Krimbas (1993).

RESULTS

In the *D. subobscura* population from Deliblato, 14 different chromosomal arrangements of the five chromosomes of the set were registered, formed by

Table 1. Gene arrangement frequencies (%), heterozygosity (HZ) and Index of free recombination in two *D. subobscura*

Gene arrangement	Deliblato (%) (n = 33)	Belgrade (%) (n = 31)
A _{st}	54.55	51.61
A ₁	45.45	41.94
A ₂		6.45
A ₂₊₈₊₉	/	/
J _{st}	24.24	41.94
J ₁	75.76	58.06
U _{st}	12.12	14.52
U ₁	/	/
U ₁₊₂	71.21	67.74
U ₁₊₂₊₃	/	/
U ₁₊₂₊₆	16.67	17.74
U ₁₊₂₊₈	/	/
E _{st}	28.79	35.48
E ₁₊₂	/	/
E ₁₊₂₊₉	18.18	25.81
E ₁₊₂₊₉₊₁₂	/	1.61
E ₈	53.03	37.10
O _{st}	36.36	46.77
O ₁	/	/
O ₅	/	/
O ₆	/	/
O ₂₂	/	/
O ₃₊₄	37.88	40.32
O ₃₊₄₊₁	19.70	11.29
O ₃₊₄₊₂	6.06	1.61
O ₃₊₄₊₆	/	/
O ₃₊₄₊₇	/	/
O ₃₊₄₊₈	/	/
O ₃₊₄₊₂₂	/	/
HZ	0.484	0.589
IFR	85.11	81.83

13 inversions. In the Belgrade population, 15 inversions were registered forming 16 different chromosomal arrangements. Table 1 shows the frequencies of chromosomal gene arrangements in both populations. Gene arrangement A₂ is present in the Belgrade population in low frequency but it is not registered in the Deliblato population. There is a considerable difference between the J_{st} and J₁

frequencies when these two populations were compared. E_{st} gene arrangement is higher in frequency in the population from Belgrade, while E₈ is more frequent in Deliblato. The complex gene arrangement E₁₊₂₊₉₊₁₂ was found in the Belgrade population but not in that of Deliblato. O_{st} and O₃₊₄ gene arrangements have higher frequency in the Belgrade population. The complex gene arrangement O₃₊₄₊₂ is present in both populations with low frequency, but somewhat more frequent in Deliblato.

Comparison of gene arrangement frequencies between the samples from Deliblato and Belgrade, by using z-test, shows significant differences only for J_{st} ($z = 3.015$; $p < 0.05$) and J₁ ($z = -3.015$; $p < 0.05$).

The G-test reveals significant differences in the distribution of gene arrangements only for the chromosome J ($G = 4.57$; $p < 0.05$) between these two populations.

The index of free recombination (IFR) and index of heterozygosity (HZ) computed for the Deliblato and Belgrade populations are also given in Table 1. The IFR values are generally higher than expected for the region. The population from Belgrade has a lower IFR value (81.83) compared to Deliblato (85.11), and to other populations from the region. Also, the value of heterozygosity (percentage of heterokaryotypes) is higher in the population from Belgrade (0.589) than in population from Deliblato (0.484).

DISCUSSION

In this study, the obtained results show that populations of *Drosophila subobscura* from ecologically and topologically distinct habitats possess a certain degree of genetic differences, probably due to their different evolutionary histories. In general, the pattern of inversion polymorphism of these analyzed *D. subobscura* populations is consistent with the hitherto observed inversion polymorphism for the area of the south-east margin of the Central European area of the species (Krimbas, 1993).

Table 2. Genetic distances (according to Prevosti, 1975) between populations

	A	K	S	D	BG	DJ	P	G	J	A	K	S	D	BG	DJ	P	G	J	
<u>A chromosome</u>										<u>J chromosome</u>									
K	0.065									0.290									
SL	0.080	0.075								0.050	0.240								
D	0.085	0.14	0.085							0.170	0.280	0.170							
BG	0.095	0.150	0.095	0.030						0.190	0.350	0.190	0.120						
DJ	0.095	0.140	0.115	0.050	0.040					0.170	0.280	0.170	0.020	0.140					
P	0.050	0.045	0.080	0.095	0.105	0.095				0.260	0.090	0.210	0.200	0.320	0.190				
G	0.200	0.175	0.200	0.285	0.295	0.285	0.210			0.430	0.290	0.400	0.570	0.590	0.570	0.380			
J	0.260	0.205	0.260	0.345	0.355	0.345	0.250	0.060		0.520	0.410	0.520	0.690	0.710	0.690	0.500	0.120		
Z	0.115	0.160	0.135	0.070	0.050	0.020	0.115	0.305	0.365	0.190	0.300	0.190	0.020	0.120	0.020	0.210	0.590	0.710	
<u>U chromosome</u>										<u>E chromosome</u>									
K	0.500									0.450									
SL	0.460	0.380								0.410	0.280								
D	0.410	0.310	0.170							0.360	0.380	0.200							
BG	0.380	0.300	0.190	0.030						0.310	0.310	0.190	0.100						
DJ	0.310	0.350	0.300	0.180	0.160					0.310	0.300	0.170	0.130	0.030					
P	0.410	0.120	0.370	0.310	0.280	0.260				0.380	0.120	0.160	0.280	0.220	0.210				
G	0.800	0.320	0.440	0.570	0.590	0.650	0.420			0.680	0.300	0.510	0.570	0.600	0.600	0.410			
J	0.820	0.410	0.520	0.690	0.710	0.760	0.500	0.230		0.570	0.410	0.610	0.690	0.720	0.710	0.510	0.140		
Z	0.310	0.340	0.290	0.170	0.150	0.060	0.240	0.640	0.740	0.360	0.400	0.250	0.210	0.130	0.110	0.290	0.700	0.800	
<u>O chromosome</u>										<u>All chromosomes</u>									
K	0.510									0.363									
SL	0.380	0.170								0.276	0.229								
D	0.260	0.320	0.210							0.257	0.286	0.167							
BG	0.210	0.320	0.200	0.070						0.237	0.286	0.173	0.070						
DJ	0.400	0.330	0.310	0.220	0.200					0.257	0.280	0.213	0.120	0.114					
P	0.400	0.140	0.110	0.220	0.220	0.230				0.300	0.103	0.186	0.221	0.229	0.197				
G	0.830	0.440	0.480	0.580	0.650	0.690	0.540			0.588	0.305	0.406	0.515	0.545	0.559	0.392			
J	0.650	0.510	0.520	0.690	0.710	0.790	0.620	0.380		0.564	0.389	0.486	0.621	0.641	0.659	0.476	0.186		
Z	0.360	0.370	0.300	0.130	0.170	0.130	0.270	0.670	0.780	0.267	0.314	0.233	0.120	0.124	0.068	0.225	0.581	0.679	

A - Apatin; K - Kamariste; S - Slankamen; D - Deliblato; BG - Belgrade; DJ - Djerdap; P - Petnica; G - Goc; J - Jastrebac; Z - Zanjic

Frequencies of gene arrangements of the chromosome A in populations from Deliblato and Belgrade are coherent with frequencies expected for the Balkan region. For chromosome J there is a discrepancy, with the standard arrangement showing a lower frequency in Deliblato, while J_1 shows frequencies higher than expected, both of which imply a more southern pattern. The U_{st} in both populations has a lower frequency than expected for this region (more southern characteristic). The frequencies of the arrangement of U_{1+2} are much higher than expected from the geographical position of localities of Deliblato and Belgrade. The complex arrangement U_{1+2+6} , which is more frequent in the Balkans than in other parts of Europe, has a frequency more similar to northern popula-

tions (values lower than expected), in both analyzed populations. The arrangement of E_8 shows a significantly higher frequency than expected (more of a south-eastern type), in both populations. The O_{st} arrangement in the population from Belgrade has a slightly higher frequency than expected (more northern in type). Both populations have frequencies of O_{3+4+2} considerably lower than expected for the Balkan region.

Genetic distances based on the differences in the frequency of chromosomal gene arrangements, calculated according to Prevosti (1975), are given in Table 2. They were calculated to quantify the difference in the inversion polymorphism between the populations from Belgrade and Deliblato, and to

compare them to several populations completely analyzed to date in the Central Balkan region: Apatin and Žanjic (Živanović et al., 2002), Kamarište (Živanović et al., 2000), Slankamen (Kalajdžić et al., 2006), Deliblato (Jelić et al., 2009), Djerdap (Živanović et al., 2003; 2007), Petnica (Anđelković et al., 1998), Goč (Anđelković et al., 2003; Savković et al., 2004; Stamenković-Radak et al., 2008) and Jastrebac (Živanović et al., 1995). The map of all the localities is shown in Fig. 1. Only populations analyzed in a relatively close time period were included for comparison, in order to avoid possible differences in chromosomal inversion polymorphism influenced by long temporal distance. The smallest distances are associated with chromosomes A and J, and the largest with U, E and O, which means that the latter contribute most to the observed distances among the compared populations. Regarding all chromosomes compared to the other populations, the most differentiated are those from Jastrebac (Živanović et al., 1995) and Goč (Anđelković et al., 2003), while between these two populations the distances are small. The populations from Apatin and Žanjic (Živanović et al., 2002) are the next most differentiated when compared with the populations of Jastrebac and Goč, respectively. The smallest distance is between Belgrade and Deliblato.

The differences in chromosomal inversion polymorphism among the ten populations are not randomly distributed. A G-test for pairwise comparisons between the populations of the central Balkans showed significant differences (Table 3) in the distribution of the chromosomal arrangement frequencies for the chromosomes J, U, E and O and for all chromosomes in total. The gene arrangements on the chromosome A are homogeneously distributed among all populations and are therefore not presented. Although the area from which populations were sampled doesn't cover a wide spatial range, considerable differentiation between the populations is evident. This implies that the pattern of inversion polymorphism of this area is shaped by several ecological factors.

The variety of the chromosomal inversion polymorphism of the *D. subobscura* populations analyzed

here links them to the region of Central and Southern Europe. The Pannonian population of Apatin (Živanović et al., 2002) is the closest to the pattern of inversion polymorphism for Central Europe due to the presence of standard gene arrangements in high frequencies and the arrangements of E_{1+2+9} and O_{3+4+1} in low frequencies, which is characteristic for the Balkans.

The general pattern of inversion polymorphism in populations from Central Balkan is more southern (Krimbas, 1993). This can be explained by the fact that chromosomal inversion polymorphism has a tendency to change to a more southern type with time. These changes could be due to climatic factors and track global climate warming and they have been observed for several *D. subobscura* populations in Europe and in both the Americas (Orengo & Prevosti, 1996; Sole et al., 2002; Balanya et al., 2003, 2006, 2009).

Latitudinal clines that are generally present throughout the distribution of the species were also observed in the studied area. The frequencies of most arrangements change gradually from north to south. The correlation coefficients between the gene arrangement frequencies and geographic latitude of the populations from Belgrade, Deliblato and several other populations from the central Balkans studied so far, are given in Table 4. The standard gene arrangements of all chromosomes and arrangement E_8 show a positive latitudinal correlation while all the other arrangements show the opposite. Obviously, the range of latitude ($45^{\circ}40'N - 42^{\circ}24'N$) in the area studied is not large enough to produce significant correlations. Significant deviation from zero is found only for the gene arrangements U_{1+2+3} and O_{3+4+8} . Nevertheless, the standard arrangements of other chromosomes also show a positive correlation with latitude, and thirteen out of fourteen non-standard arrangements show a negative correlation with latitude. That is in accordance with the latitudinal variation of the chromosomal inversion polymorphism of *D. subobscura* observed throughout Europe (Krimbas, 1993) and in the colonizing populations from America (Prevosti et al., 1990). It is evident that some ecological factor associated

Table 3. G-test for chromosomal arrangement frequency in pairwise comparisons (p-values are corrected for multiple comparisons)

	Chromosome J		Chromosome U		Chromosome E		Chromosome O		all chromosomes	
BG/G	10.942	*					33.729	***	64.833	***
BG/S										
BG/Ž									59.146	***
BG/K	12.721	*	23.676	***					71.842	***
BG/P	15.444	***	25.155	***					69.506	***
BG/D										
BG/A			36.488	***	23.322	**			71.460	***
BG/J									42.838	*
G/S			18.516	**	30.530	***			80.295	***
G/Ž					35.735	***	28.299	**	83.812	***
G/K			15.932	*			42.610	***	66.228	***
G/P			22.463	***			36.707	***	72.858	***
G/D					21.754	***	17.995	*	53.098	***
G/A	11.530	*	77.540	***	68.790	***	88.302	***	246.164	***
G/J							23.992	*	42.438	*
S/Ž							23.695	*	64.028	***
S/K			36.205	***					89.192	***
S/P	10.911	*	34.038	***					83.549	***
S/D										
S/A			47.321	***	41.125	***	36.441	***	128.218	***
S/J			23.831	***	24.054	***			63.042	***
Ž/K			14.357	*	21.543	**			59.103	**
Ž/P										
Ž/D					28.498	***			68.055	***
Ž/A			43.072	***	31.039	***	47.536	***	130.477	***
Ž/J					25.016	***	23.090	*	61.319	***
K/P										
K/D			27.190	***	25.927	***	22.623	*	86.652	***
K/A	13.280	*	48.434	***	46.189	***	55.602	***	165.737	***
K/J							25.203	*		
P/D			28.928	***	18.002	*			75.834	***
P/A	16.228	***	40.475	***	33.168	***	40.426	***	132.673	***
P/J							30.542	*		
D/A			43.774	***	33.245	***			99.018	***
D/J			17.923	*	20.633	***			53.766	***
A/J			60.168	***	47.218	***	55.480	***	169.839	***
DJ/G			23.312	**			54.217	***	95.288	***
DJ/S			22.140	**			35.563	***	51.737	***
DJ/Z										
DJ/K			20.051	*					47.188	*
DJ/P			19.375	*						
DJ/D			18.119	*			29.007	*	63.780	***
DJ/A			42.632	***	23.332	**	49.188	***	108.477	***
DJ/J			21.464	*			37.513	***	50.024	*
DJ/BG			16.403	*					51.790	*

A - Apatin; K - Kamariste; S - Slankamen; D - Deliblato; BG - Belgrade; DJ - Djerdap; P - Petnica; G - Goc; J - Jastrebac; Z - Zanjic

with latitude is shaping the overall pattern of chromosomal inversion polymorphism, but it is difficult to completely eliminate the possible role of historical events. It is hypothesized that one of the refugee areas for *D. subobscura* during the last glaciations might have been the southern Balkans and Asia Minor. A major passage of recolonization of *D. subobscura* would have followed starting from those areas to the north and the west of Europe. Although the standing geographic patterns of distribution of inversion polymorphism could only mirror historical processes, the formation of N-S clines is better attributed to the action of selective forces. Resistance to the cold, or any other factor related to temperature or latitude, more than differential migration ability, would be a likely candidate (Menozzi and Krimbas, 1992; Santos et al. 2005).

The population from the Botanical Garden, situated in a central urban zone of Belgrade, is not highly differentiated if compared to the other populations, especially geographically close ones like that from Deliblato, although it is under strong anthropogenic influence and inhabits a highly polluted environment. The only distinction of this population is the lowest IFR value when compared to all the other populations analyzed in region of the central Balkans. If low IFR values correspond to the high values of mean heterozygosity (Prevosti et al., 1984) then the polymorphism (heterozygosity) is highest in the urban population from Belgrade. This population also has a higher percent of heterokaryotypes when compared to Deliblato, so the heterozygosity is higher at a chromosomal level, as well. Da Cunha and Dobzhansky (1954) and da Cunha et al. (1959) showed a positive correlation between environmental complexity and inversion heterozygosity. Urban areas are considered as environments that are unpredictable and prone to sudden fluctuations of ecological factors. Also, the greatest variety of microhabitats is characteristic for this type of environment. Considering all of this, a higher degree of heterozygosity is expected in populations that inhabit urban environments compared to populations from other habitats, though it doesn't have to be reflected in heterozygosity at the

Table 4. Correlation coefficients (r) between gene arrangements frequencies and latitude in *D. subobscura* populations

Gene arrangement	r	p
A _{st}	0.611	0.060
A ₁	-0.422	0.224
A ₂	-0.276	0.440
J _{st}	0.337	0.342
J ₁	-0.337	0.342
U _{st}	0.566	0.088
U ₁	-0.027	0.941
U ₁₊₂	-0.168	0.643
U ₁₊₂₊₃	-0.766	0.010
U ₁₊₂₊₆	-0.238	0.509
E _{st}	0.413	0.235
E ₁₊₂	-0.411	0.238
E ₁₊₂₊₉	-0.364	0.301
E ₈	0.127	0.728
O _{st}	0.592	0.071
O ₃₊₄	-0.366	0.298
O ₃₊₄₊₁	-0.393	0.261
O ₃₊₄₊₂	-0.615	0.059
O ₃₊₄₊₈	-0.733	0.016

chromosomal level. According to several authors, marginal *Drosophila* populations are generally characterized by a lower chromosomal polymorphism if compared to central populations (Townsend, 1952; Dobzhansky, 1957; Da Cunha et al., 1959; Carson, 1955, 1956, 1959; Prevosti, 1964; Sperlich, 1971). A hypothesis proposed by Fontdevila (1992) tries to explain the high degree of chromosomal polymorphism in marginal populations of *Drosophila*, and according to it, marginal populations are more subject to genomic stress capable of promoting the mobilization of transposable elements, recognized by their ability to yield chromosome rearrangements. Although the population from Botanical Garden is regarded as extremely marginal and with the ecological characteristics specific for the urban environment, it has a considerable number of chromosomal variants, but

the level of chromosomal inversion polymorphism in this population is not higher than in the other populations from localities relatively undisturbed by anthropogenic activities.

The observed differences between the populations can be interpreted as a consequence of selection interacting with historical factors related to geographical conditions. All these factors determine the degree of genetic isolation among populations. However, any consistent difference among *Drosophila* populations is observed only over longer distances. The strong dispersal capacity of the fruit flies is the reason for such long-distance variations (David and Capy, 1988; Serra et al., 1987; Pascual et al., 2000). It can be concluded that the population of *D. subobscura* from the Botanical Gardens does not represent a marginal population of this species, and that the selective pressure that arises from environmental deterioration caused by human activity and urbanization is not so strong, or is at least tolerable for the species and this specific population.

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ИНВЕРЗИОНИ ПОЛИМОРФИЗАМ ПОПУЛАЦИЈА *DROSOPHILA SUBOBSCURA* ИЗ УРБАНЕ И НЕ-УРБАНЕ СРЕДИНЕ

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Урађена је анализа инверзионог полиморфизма у популацијама *Drosophila subobscura* пореклом из урбане средине, локалитет Ботаничка башта у Београду, и са локалитета у Делиблату, Србија, који није под јаким антропогеним утицајем. Поређењем инверзионог полиморфизма ове две популације међусобно, као и са другим популацијама у региону, испитане су карактеристике и динамика

овог типа полиморфизма на централном Балкану. Без обзира што се популација из Београда налази под јаким антропогеним утицајем, она не показује смањење броја инверзија и није значајно диференцирана у поређењу са географски блиским популацијама. Може се закључити да селективни притисак узрокован загађењем није јак или да је популација прилагођена на загађење.