GENETIC ANALYSIS IN *Drosophila melanogaster* NATURAL POPULATIONS COLLECTED FROM DIFFERENT ECOSYSTEMS SUBJECTED TO ABIOTIC STRESS

CHELU Cristina, CORNEANU Mihaela, BUTNARU Gallia

Abstract. We used Random amplified polymorphic DNA (RAPD) to analyze DNA polymorphisms for 9 *Drosophila melanogaster* populations collected from salty soils, radioactivity and arid zones from Romania. In this study we used 10 RAPD primers (10 bp) in order to determine genetic distance between our collected populations from different ecosystems. Using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) we obtained a phylogenetic tree which divided our *D. melanogaster* populations in two groups related to the specific area collection. *D. melanogaster* Socodor has proved to be the oldest, being grouped with the wild type, Oregon. We found two unique bands in *D. melanogaster* Peşteana and Plopşoru populations, both of them were collected from mining areas. The genetic distance is small between *D. melanogaster* populations according with the phenotype traits and life span.

Keywords: *Drosophila melanogaster*, populations, RAPD, genetic distance, phylogenetic tree.

Rezumat. Analize genetice la populaţiile naturale de *Drosophila melanogaster* colectate din diferite ecosisteme supuse stresului abiotic. Pentru analiza ADN-ului polimorfic la 9 populaţii de *Drosophila melanogaster* colectate de pe soluri de sărătură, radioactivitate şi zone aride din România s-a utilizat metoda amplificării ADN-ului polimorfic la întâmplare (RAPD). În acest studiu s-au utilizat 10 primeri (10 pb) pentru determinarea distanţei genetice dintre populaţiile colectate din diferite ecosisteme. Prin metoda UPGMA s-a obţinut un arbore filogenetic care împarte populaţiile de *D. melanogaster* în 2 grupe în conformitate cu specificul locului de colectare. *D. melanogaster* Socodor s-a dovedit a fi cea mai veche, fiind grupată cu tipul sălbatic, Oregon. S-au observat 2 benzi unice la populaţiile *D. melanogaster* Peşteana şi Plopşoru, ambele au fost colectate din zone cu activitate minieră. Distanţa genetică dintre populaţiile de *D. melanogaster* este mică, conform cu caracterele fenotipice şi ciclul de viaţă.

Cuvinte cheie: *Drosophila melanogaster,* populaţii, RAPD, distanţă genetică, arbore filogenetic.

INTRODUCTION

Natural populations are constantly exposed to challenging environments and it is necessary for the organism to buffer this environmental variation to maintain the cellular homeostasis and high performance across environmental. The stress response and heat shock proteins are important for this buffering in relation to stress resistance and adaptation to the environment under some conditions (SORENSEN et al., 2003). All organisms are strongly affected by their surrounding environment, and the environmental factors play an important part in shaping ecology and evolution of biological systems. Environmental stress is especially important at many levels of biological organization (HOFFMANN & PARSONS, 1997; HOFFMANN & HERCUS, 2000). In this context environmental stress is regarded as an "environmental factor causing a change in a biological system, which is potentially injurious" (HOFFMANN & PARSONS, 1991) and which has some fitness consequences (BIJLSMA & LOESCHCKE, 1997). Spatial and temporal variations, which predominate in nature, is of prime importance in maintaining genetic diversity in natural populations. This ecological genetic pattern is true, because different genotypes display varying fitness in variable environments and stresses. Recombination frequencies and mutation rates tend to increase under stressful conditions (HOFFMANN & PARSONS, 1991; KOROL, 1999). Changes in vegetation also lead to changes in the local microclimate. The variation in the actual local temperatures is even higher than that of the air temperatures as recorded by standard measurement techniques. Vegetation that is more open causes higher light intensity on the ground. Both temperature and openness affect humidity and the air is near saturation throughout the day in closed-canopy forest but fluctuates greatly in more open vegetation (WALTER, 1984 cit. VAN DER LINDE & SEVENSTER, 2006). The effect of temperature has been studied in different species of *Drosophila* on both adult and preadult characters. Interspecific competitions of larvae have shown to be influenced by temperature in *Drosophila* (FOGLEMAN & WALLACE, 1980; BUDNIK & BRNCIC, 1983; RICCI & BUDNIK, 1984). A combination of genomics, proteomics and metabolomics will further elucidate the effects of stress on expression patterns at the DNA, RNA and protein levels and the effect on metabolism (LOESCHCKE et al., 2004; MALMENDAL et al., 2006).

The aim of the present study was to determine phenotypic and molecular polymorphism among several *Drosophila melanogaster* natural populations collected from salty soils, mining areas and aridity zones from Romania.

MATERIAL AND METHODS

***Drosophila melanogaster* populations.** In our study we used 9 populations of *Drosophila melanogaster* which were collected from different areas of Romania, including polluted zones as it follows: Socodor (solonchaks and steppe vegetations, plain area), Tg-Jiu, Peşteana, Plopşoru and Turceni (submountain hilly area, mines activity), Bucovăţ (forest, natural radioactivity), Giubega and Moţăţei (sand dunes and arid zones, plain area), Şag (unspecific pollution) and as control we used the wild type, Oregon. The name of our population comes from the collection areas. Collection was done using traps in areas of interest on shaded places, in the morning. Traps were made by glass jars with perforated cover and the attractant was represented by fermented fruit, especially bananas and the trap was collected in

the evening. During the analyses, the populations of *Drosophila melanogaster* were maintained in laboratory conditions (25 ˚C) using a corn-meal, yeast and sugar medium. The experiment was conducted in two repetitions, at 25˚C. We used adult individual, 1-4 days old, sex-ratio 1:1. Observations were made for 28 days until the last individuals hatched out.

DNA extraction. We chose randomly 20 flies from each populations and we isolated DNA by rapid and small isolation method after Steller protocol (cit. by RUBIN, 1990). The concentration of extracted DNA was measured at spectrophotometer and the purity was calculated by ratio of absorbance at 260 nm and that of 280 nm. The isolated DNA was diluted at 50 ng/µl.

RAPD analysis. In order to establish genetic polymorphism among our collected populations of *Drosophila melanogaster* we used random amplified polymorphic DNA (RAPD) technique, based on DNA markers. In RAPD reactions we used 10 ologonucleotide primers (Biosearch Techologies) with sequences: P1 5'(TGC-GGG-AGT-G)3', P3 5' (AAG-AGC-CCT-A)3', P4 5'9GGC-TTG-GCG-A)3', P5 5'(CAC-TGG-CCC-A)3', P7 5'(TGG-TCG-GGT-G)3', P8 5'(CTA-AGC-GCA)3', P9 5'(TTG-CTG-GGC-G)'3, P11 5'(CCG-CTG-GAG-C)3', P15 5'(GCT-CCC-CCA-C)3', P16 5'(TTG-CTG-GGC-G)3'. PCR mixture was performed in a 25 µl final volume, containing the following components: 50 ng/µl DNA, 1.5 unit of Taq DNA Polymerase (Fermentas), Dream Taq Buffer (Fermentas), 25 mM $MgCl_2$ (Fermentas), 25 mM dNTPs (Fermentas), 10 µM primer and H_2O distilled water until final volume. PCR reactions were run in a DNA Thermocyler (Biorad) using the next program: 3 min denaturation at 94°C, followed by 36 cycles of 1 min at 94°C, 1 min at 36°C, extension was done at 72°C for 2 min and final extension at 72°C for 7 min. The PCR products were migrated in agarose gel (1.2%) by electrophoresis in TBE buffer (5X), separating them according to their molecular weight. Amplified DNA fragments were stained with ethidium bromide and visualization of DNA bands and photography was done with UV Vilber Lourmat. Images (photos) obtained were processed in Microsoft Office Power Point.

Data analysis. The present bands on the agarose gel were scored with 1 and the absence was noted with 0. We take into account only the bands well reproduced in both repetitions in order to obtain a binary matrix. The genetic similarity was calculated based on Jaccard's coefficient. The complement of Jaccard similarity coefficient represents the genetic distance between the populations of *D. melanogaster*, based on these results it has been achieved the matrix of distance. The data obtained was used to construct a dendrogram based on UPGMA algorithm (Unweighted Pair Group Method of Arithmetical Averages) by SAITOU & NEI (1987). Statistical analysis regarding body size were performed by measuring 5 female, 5 males and also 5 larvae and 5 pupae form each population. For the life cycle and sex ratio we counted the emerging flies every day. We also noticed the number of non emerged individuals in pupa stage of development.

RESULTS AND DISCUSSION

Morphological description of *Drosophila melanogaster* populations. In our study we used 9 natural populations of *Drosophila melanogaster* collected from different polluted areas and standard type, Oregon, for control. After collection we have analyzed the phenotype of each population regarding eye color, number of abdominal segments, abdomen and wing shape (Fig. 1). There was no major difference between collected populations of *Drosophila melanogaster*.

Adult. Our collected populations from different ecosystems have red eyes, with no differences compared with the standard type Oregon. The body is yellow in natural populations of *D. melanogaster*, and black striped abdomen, slightly on females, and round on males, the last segment being black. Our populations of *D. melanogaster* are characterized by body size (Fig. 1) between 0.31±0.00 and 0.34±0.01 cm for females and 0.26±0.01 to 0.30±0.00 cm among males.

Intra-population variability of body size is small, gently is detaching *D. melanogaster* Plopşoru population, followed by *D. melanogaster* Moțăței in case of females and in males the population *D. melanogaster* Peşteana followed by Bucovăț, Turceni and Plopşoru populations. Compared with the wild type Oregon (0.34±0.00 cm) females, the body size of the populations of *D. melanogaster* Târgu-Jiu and Plopşoru presents low values like 0.31 cm and 0.32 cm. Regarding body size in males, only populations of *D. melanogaster* Socodor and Şag have the same average of value (0.30±0.00 cm), in the other populations individuals being smaller, with the average sizes ranging up to 0.26±0.01 cm in *D. melanogaster* Bucovăț population.

By the transparency of the abdomen we observed that the ovaries are white and testes are yellow (Fig. 2), sexual dimorphism in *Drosophila melanogaster* being well defined. Our natural populations of *D. melanogaster* have normal wings (Fig. 3) as the control (Oregon). The form differs slightly from population to population.

Larva. The color of larva is white in all *Drosophila melanogaster* collected populations (Fig. 4). Regarding larvae dimensions, the highest value (0.48±0.01 cm) was determined for *D. melanogaster* Turceni population and the lowest intra-populational variability when was compared with wild type, Oregon which showed the highest variability of the larvae size with an average of 0.38±0.02 cm. Larvae with the smallest dimensions belong to *D. melanogaster* Bucovăț population (0.37±0.01 cm). *D. melanogaster* Sag population presents a medium variability which results from a non-specific environmental pollution. Inter-populational variability is small, as the obtained percentage was 7.37%.

Pupa. Pupa color is yellow-brown in *D. melanogaster* populations and slightly red in *D. melanogaster* Giubega and Bucovăț. The size of pupae (Fig. 4) varies from 0.30±0.00 cm in *Drosophila melanogaster* Socodor and Plopşoru populations and Oregon, to 0.34±0.02 cm in *D. melanogaster* Giubega. The highest intra-populational variability in pupa stage was determined for *D. melanogaster* Giubega population and the lowest for Oregon.

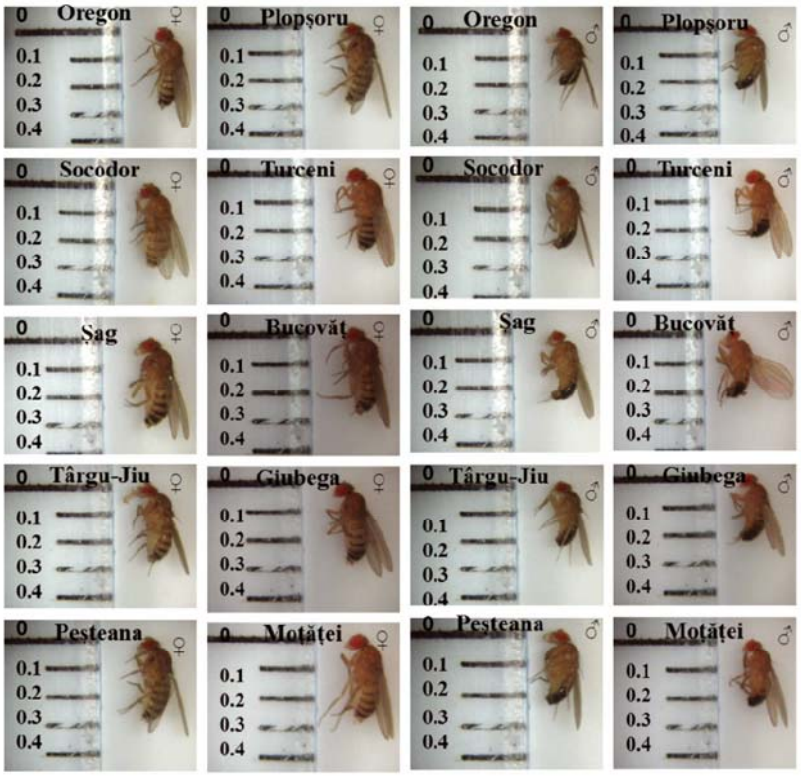

Figure 1. Morphological traits in natural populations of *D. melanogaster*, in the left panel we presented females and males in the right panel (original).
Figura 1. Caracterele morfologice la populaţiile naturale de *D. melanogaster*, în panelul din stânga sunt prezentate femelele şi masculii în panelul din dreapta (original).

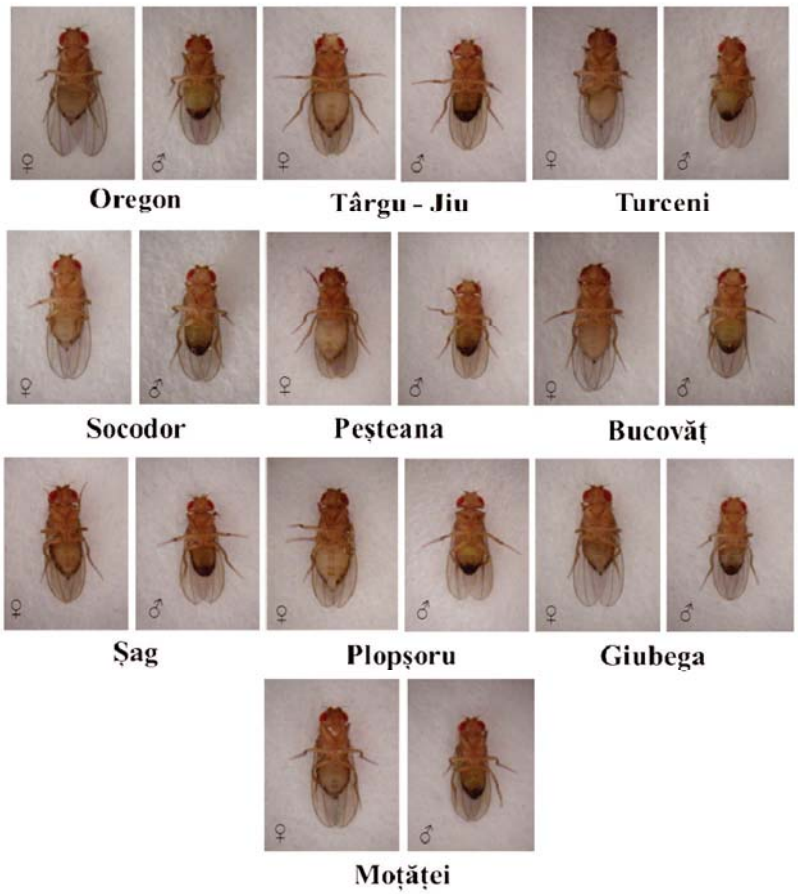

Figure 2. Ventral part of the body in *D. melanogaster* natural populations (original).
Figura 2. Partea ventrală a corpului la populaţiile naturale de *D. melanogaster* (original).

Table 1. Phenotypic characters (body size in females and males, larva and pupa size) in *Drosophila melanogaster* natural populations. / Tabel 1. Caracterele fenotipice (dimensiunea corpului la femele şi masculi, dimensiunea larvei şi a pupei) la populaţiile naturale de *Drosophila melanogaster*.

Population	Statistical parameters															
	Body size ♀				Body size ♂				Larva size				Pupa size			
	$x \pm s_x$	s^2	s%	s	$x \pm s_x$	s^2	s%	s	$x \pm s_x$	s^2	s%	s	$x \pm s_x$	s^2	s%	s
Oregon	0.34±0.00	0.00	2.33	0.01	0.30±0.00	0.00	1.43	0.00	0.38±0.02	0.00	13.94	0.05	0.30±0.00	0.00	1.34	0.00
Socodor	0.35±0.01	0.00	3.31	0.01	0.30±0.00	0.00	2.48	0.01	0.43±0.01	0.00	4.94	0.02	0.30±0.00	0.00	1.66	0.00
Şag	0.36±0.00	0.00	1.76	0.01	0.30±0.00	0.00	2.11	0.01	0.42±0.02	0.00	11.66	0.05	0.33±0.01	0.00	8.14	0.03
Tg-Jiu	0.31±0.00	0.00	2.61	0.01	0.29±0.00	0.00	1.37	0.00	0.45±0.02	0.00	8.10	0.04	0.31±0.00	0.00	3.18	0.01
Peşteana	0.34±0.00	0.00	2.19	0.01	0.28±0.01	0.00	9.86	0.03	0.45±0.01	0.00	7.05	0.03	0.31±0.00	0.00	3.53	0.01
Plopşoru	0.32±0.01	0.00	7.19	0.01	0.28±0.01	0.00	6.10	0.02	0.42±0.01	0.00	5.49	0.02	0.30±0.00	0.00	2.51	0.01
Turceni	0.34±0.00	0.00	2.86	0.02	0.28±0.01	0.00	6.10	0.02	0.48±0.01	0.00	4.72	0.02	0.32±0.01	0.00	5.87	0.02
Bucovăţ	0.34±0.01	0.00	3.57	0.01	0.26±0.01	0.00	6.81	0.02	0.37±0.01	0.00	8.39	0.03	0.31±0.01	0.00	6.21	0.02
Giubega	0.34±0.01	0.00	3.57	0.01	0.28±0.01	0.00	5.42	0.01	0.45±0.01	0.00	5.78	0.03	0.34±0.02	0.00	13.02	0.04
Moţăţei	0.33±0.01	0.00	4.98	0.02	0.28±0.00	0.00	1.72	0.00	0.43±0.01	0.00	5.30	0.02	0.32±0.01	0.00	5.34	0.02
$x \pm s_x$	0.34±0.01				0.29±0.00				0.43±0.01				0.31±0.01			
s^2	0.00				0.00				0.00				0.00			
s%	3.99				4.23				7.37				4.08			
s	0.00				0.04				0.03				0.05			

Table 2. Larva motility (cm) in *D. melanogaster* natural populations. / Tabel 2. Motilitatea larvei (cm) la populaţiile naturale de *D. melanogaster*.

Statistical parameters	Oregon	Socodor	Şag	Tg - Jiu	Peşteana	Plopşoru	Turceni	Bucovăţ	Giubega	Moţăţei
$x \pm s_x$	2.85±0.04	3.05±0.46	3.30±0.00	2.10±0.07	3.90±0.14	1.80±0.50	2.60±0.43	3.20±0.57	3.25±0.04	2.60±0.43
s^2	0.00	0.42	0.00	0.01	0.04	0.49	0.36	0.64	0.00	0.36
s%	1.75	21.31	0.00	4.76	5.13	38.89	23.08	25.00	1.54	23.08
S	0.05	0.65	0.00	0.10	0.20	0.70	0.60	0.80	0.05	0.60

Table 3. Pupa mortality in *D. melanogaster* natural populations. / Tabel 3.Mortalitatea pupelor la populaţiile naturale de *D. melanogaster*.

Statistical parameters	Oregon	Socodor	Şag	Tg - Jiu	Peşteana	Plopşoru	Turceni	Bucovăţ	Giubega	Moţăţei
$x \pm s_x$	15.00±9.22	1.50±1.06	0.00±0.00	8.00±0.71	1.50±0.35	2.00±0.71	5.50±3.90	4.50±1.06	3.00±0.00	6.50±1.77
s^2	169.00	2.25	0.00	1.00	0.25	1.00	30.25	2.25	0.00	6.25
s%	86.67	100.00	-	12.50	33.33	50.00	100.00	33.33	0.00	38.46
S	13.00	1.50	0.00	1.00	0.50	1.00	5.50	1.50	0.00	2.50

Table 4. Livestock (average of 2 repetitions) in *D. melanogaster* natural populations. / Tabel 4. Prolificitatea (media a 2 repetiţii) la popuaţiile naturale de *D.melanogaster*.

Population	Oregon	Socodor	Şag	Tg - Jiu	Peşteana	Plopşoru	Turceni	Bucovăţ	Giubega	Moţăţei
	116.00±39.72	145.00±29.79	131.50±93.26	146.50±2.48	245.50±21.63	33.00±17.73	131.50±14.54	153.50±58.51	200.00±3.55	80.50±2.48

Table 5. Sex-ratio (♀: ♂) in *D. melanogaster* natural populations. / Tabel 5. Sex-raţio (♀: ♂) la populaţiile naturale de *D. melanogaster*.

Sex-ratio ♀: ♂	Oregon	Socodor	Şag	Tg - Jiu	Peşteana	Plopşoru	Turceni	Bucovăţ	Giubega	Moţăţei
	1.06:0.94	0.98:1.02	0.96:1.04	0.98:1.02	0.99:1.01	0.90:1.10	0.99:1.01	0.88:1.12	0.97:1.03	1.04:0.96

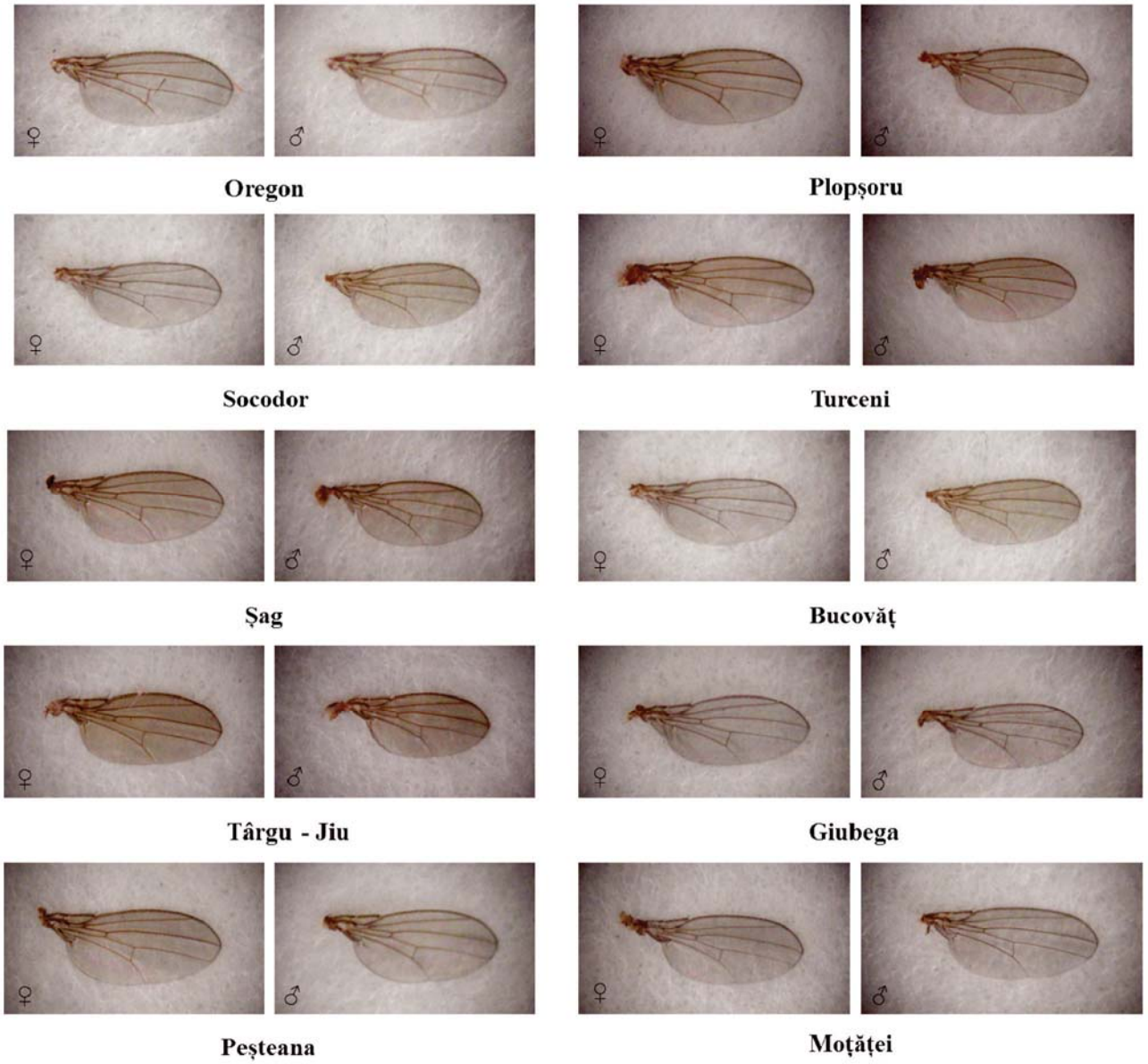

Figure 3. Wings shape in *Drosophila melanogaster* natural populations collected from salty soils, mining and arid areas (original).
Figura 3. Forma aripii la populațiile naturale de *Drosophila melanogaster* colectate de pe soluri sărăturate, zone cu activitate minieră şi ariditate (original).

Life cycle in *Drosophila melanogaster* populations. *Drosophila melanogaster* is a small insect with complete metamorphosis including all steps of development: egg, larva, pupa and imago (adult). Physical and chemical factors can influence the development of insects. Temperature, salt contained in soils or the presence of certain chemical elements represent factors included in the categories listed above which leads to changes in *Drosophila* life cycle. In Fig. 5 it is presented the life cycle of the *D.melanogaster* populations collected (average of two repetitions). Life cycle average was 9.90±0.09 days, and in case of *D. melanogaster* Plopşoru the imago stage occurs after 9 days. In wild type, Oregon, the life cycle takes 10 days.

The life span in *Drosophila melanogaster* populations collected from mining and arid areas had 10 days. We observed also the motility of larvae (Table 2). The lowest level was obtained for *D. melanogaster* Plopşoru (1.80 ± 0.50), close followed by *D. melanogaster* Târgu-Jiu population (2.10 ± 0.07). The best mobility was seen for the *D.melanogaster* Peşteana population (3.90 ± 0.14). All of these three populations were collected from closely areas, and were characterized by mining pollution.

In terms of prolificacy (Table 4), *D. melanogaster* Peşteana has proved to be the most prolific population (245.50±21.63 individuals) and had the lowest mortality (1.50 ± 0.35). This is followed by *D. melanogaster* Giubega population (200.00±3.55 individuals) and respectively *D. melanogaster* Tg-Jiu and Socodor with 146.50±2.48 and 145.00±29.79 individuals. The population with the lowest prolificacy was *D. melanogaster* Plopşoru (33.00±17.73 individuals).

The lowest level of mortality (Table 2) was observed in the case of *D. melanogaster* Şag population (0.00 ± 0.00) compared with the wild type for which was found the highest mortality (15.00 ± 9.22). Calculating sex-ratio (Table 5), we obtained a report easily dominated by males, except *D. melanogaster* Moțăței population and the wild type.

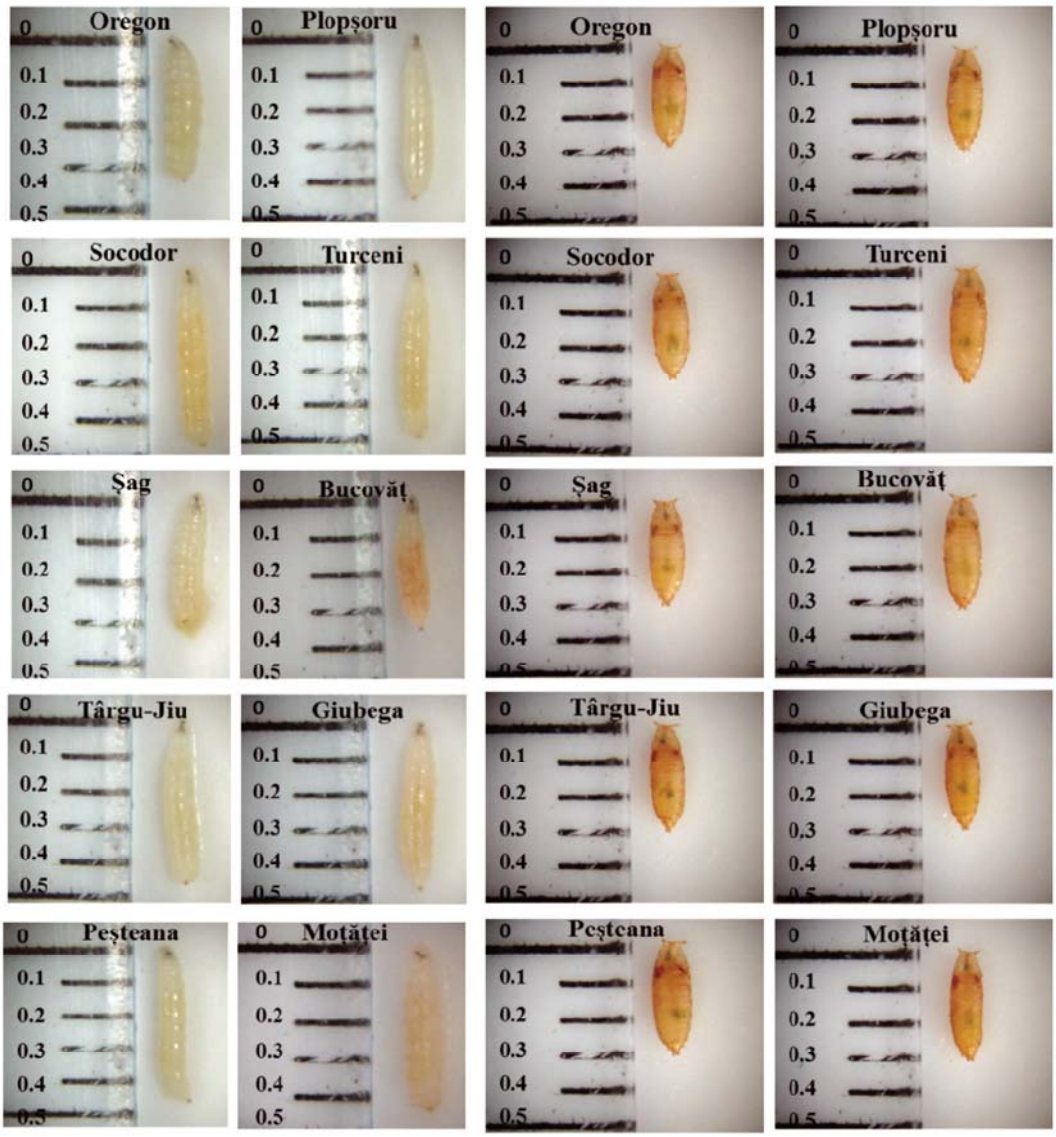

Figure 4. Larva and pupa traits in *Drosophila melanogaster* natural populations (original).
Figura 4. Caracteristicile larvei şi pupei la populațiile naturale de *Drosophila melanogaster* (original).

Molecular polymorphisms in *Drosophila melanogaster* populations by RAPD technique

RAPD is a rapid and inexpensive method used in polymorphism determination and genetic analysis of populations. Analyses presented in this paper were carried out in two repetitions and we have considered only bands that have been faithfully reproduced in both repetitions with the same intensity in agarose gel (1.2%) for all populations. For each oligomer we calculated the total number of bands, constant number of bands (bands present in all genotypes) and variable number of bands (bands that are found only in some populations). In our study we used 10 primers, oligomer 11 with sequence 5'(CCG-CTG-GAG-C)3' gave no amplification in the second repetition and for this reason it was not taken into account. For the other 9 oligomers we obtained PCR products with molecular weight ranging from 4000 bp to100 bp.

The highest total number of bands was obtained for primer 16 (18 bands) and primer 8 gave only three bands, the average of bands was 10.66. Oligomer 3 with sequence 5'(AAG-AGC-CCT-A)3' generated the largest polymorphism, 80% , and the lowest polymorphism was observed in the oligomer 16, only 11%. Overall we obtained a polymorphism of 40.24% compared with the wild type (Oregon).

Unique bands were observed in the molecular profile of *Drosophila melanogaster* Plopşoru (Fig. 8) and Peşteana (Fig. 6) populations, both belonging to areas with mining activity. Also, the wild type, Oregon has a unique band with a weight of about 2100 bp, very close to the unique band seen in *D. melanogaster* Peşteana population (2150 bp). Based on

the 96 total number of bands was constructed the matrix distance, by calculating the Jaccard coefficient for each two pairs of the populations. Present bands (amplified) were scored by 1, and the absence by 0.

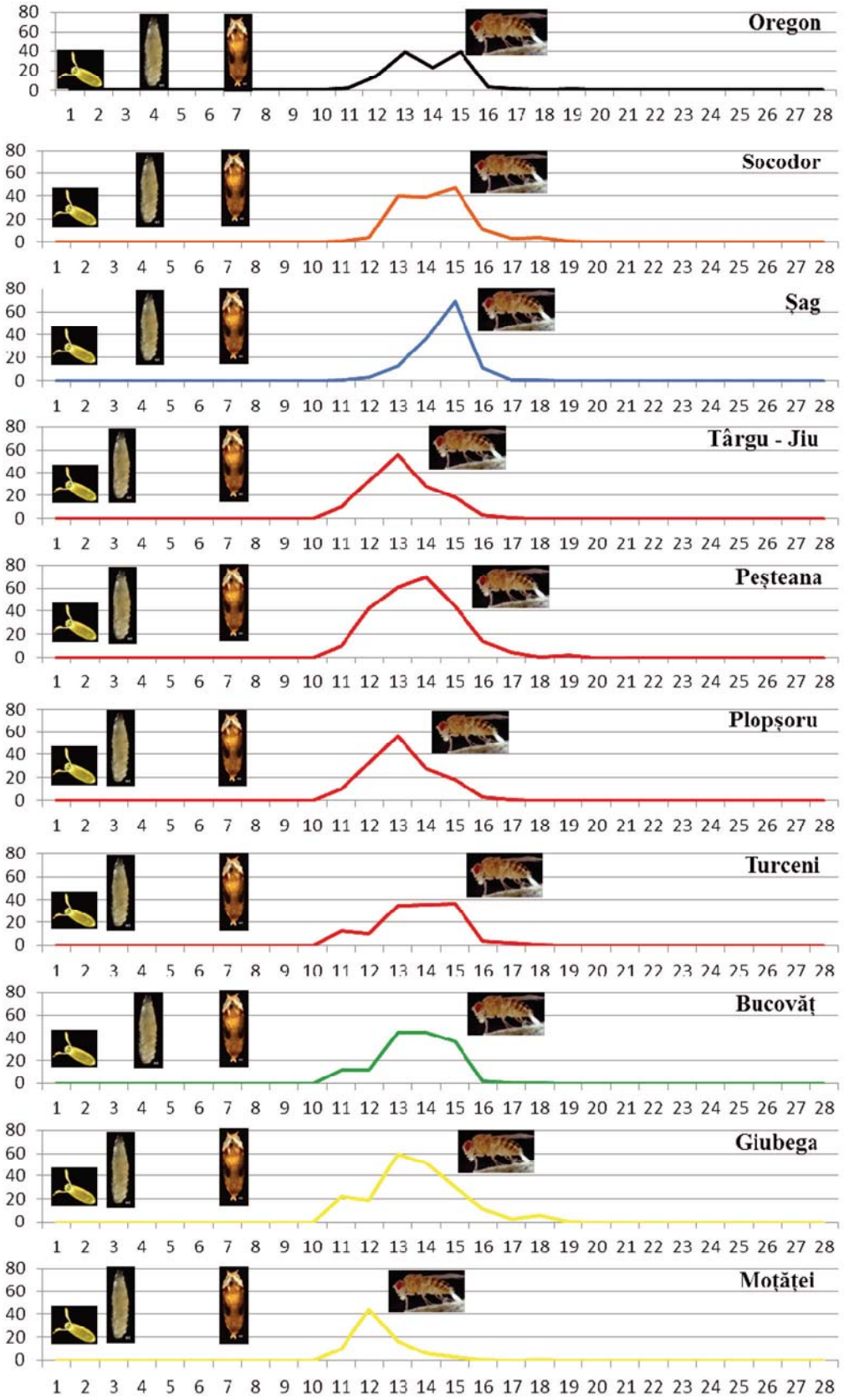

Figure 5. Life cycle in *D. melanogaster* natural populations collected from salty soils, mining areas and arid areas.
Figura 5. Ciclul de viaţă la populaţiile naturale de *D. melanogaster* colectate de pe soluri sărăturate, zone cu activitate minieră şi zone aride.

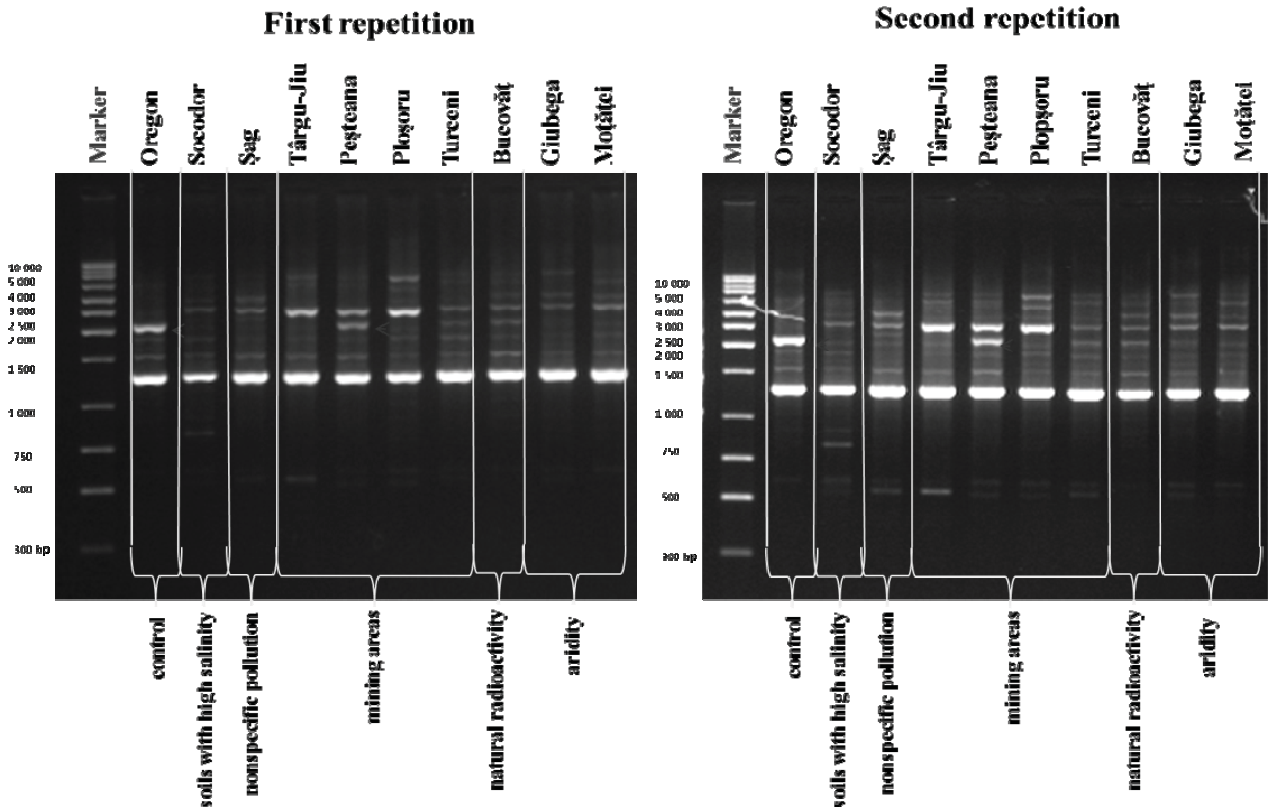

Figure 6. Molecular profile in *D. melanogaster* populations with primer P3 (red arrow - unique band).
Figura 6. Profilul molecular la populațiile de *D. melanogaster* cu primerul P3 (săgeata roşie - bandă unică).

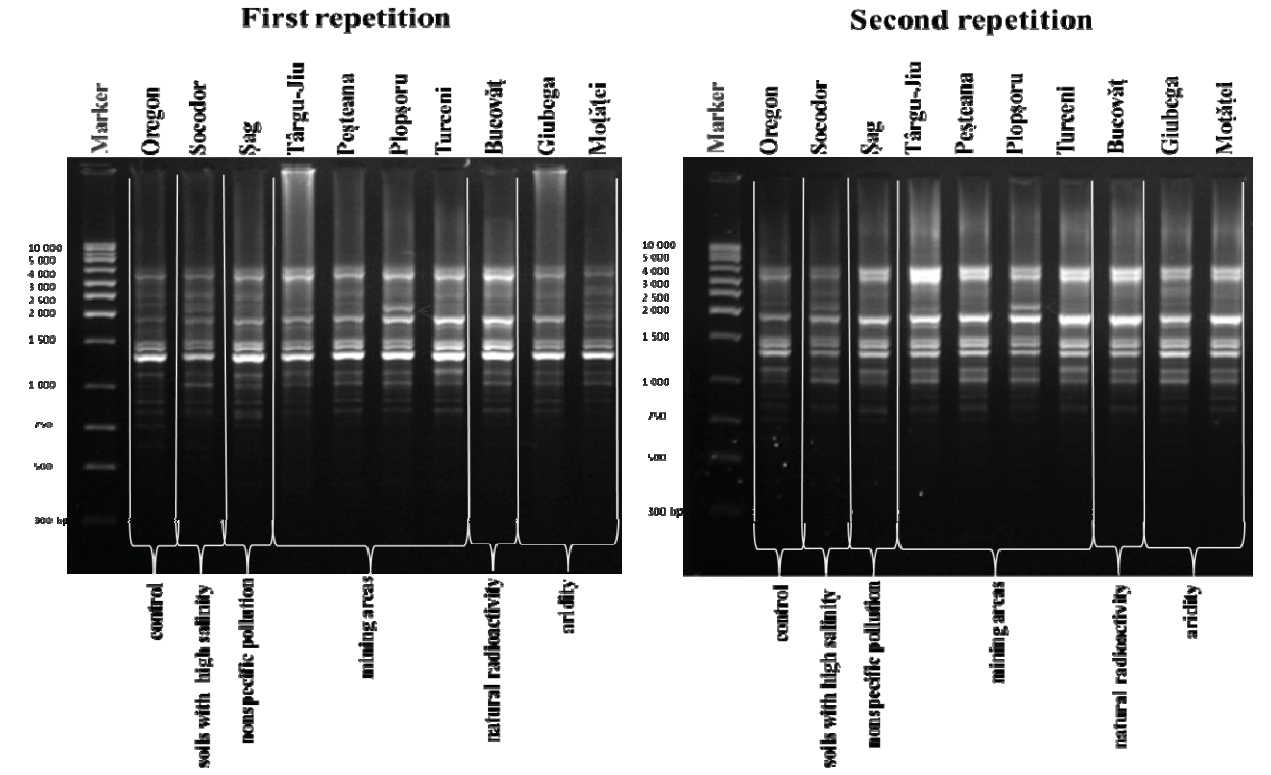

Figure 7. Molecular profile in *D. melanogaster* populations with primer P4 (red arrow - unique band).
Figura 7. Profilul molecular la populațiile de *D. melanogaster* cu primerul P4 (săgeata roşie - bandă unică).

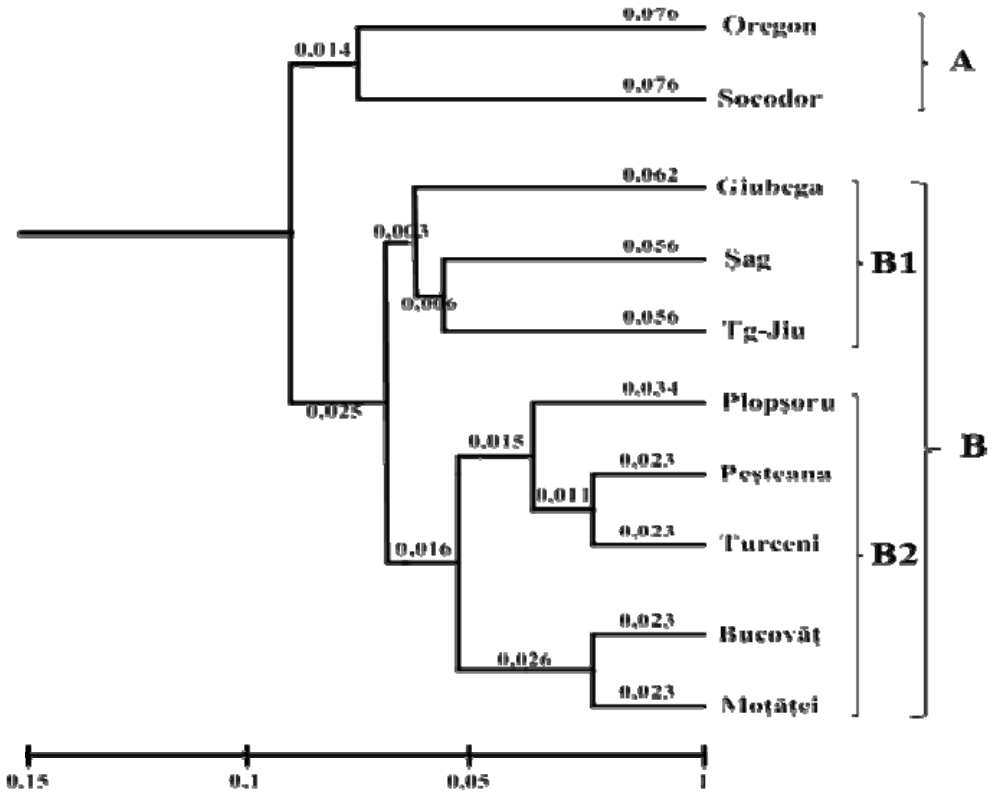

Figure 8. Dendrogram for *D. melanogaster* natural populations based on RAPD results.
Figura 8. Dendrograma pentru populaţiile naturale de *D. melanogaster* pe baza rezultatelor RAPD.

The dendrogram presented above shows two main groups, noted A and B. Group A contains wild type, Oregon and *D. melanogaster* Socodor. Group B is divided in two subgroups: one subgroup (B1) consists of *D. melanogaster* Giubega, Şag and Socodor populations and the second subgroup consists in the following populations of *D. melanogaster*: Plopşoru, Peşteana and Turceni, and respectively Bucovăţ and Moţăţei populations. In our comparative study between the wild type, Oregon, and the nine populations of *D. melanogaster* collected from different ecosystems we can notice that the nearest genetically populations are *D. melanogaster* Peşteana and Turceni populations and also *D. melanogaster* Bucovăţ and Moţăţei with the lowest genetic distance (0.023). Generally, genetic similarity values among populations were small. The root of the populations tree is positioned between this two groups, genetically group A being the oldest. The percentage of polymorphism obtained, 40.24%, shows a medium polymorphism, which can be explained by the fact that the flies could not be preserved immediately after collection.

Physiological acclimatization is a form phenotypic plasticity, by which an organism can adjust its metabolism in acute response in order to cope with the altered environmental conditions, for example, environmental stress, such as heavy metal toxicity or chemicals, and osmolarity changes. The ability to adapt to changing conditions will depend on both how well an individual can adjust to the new conditions (BAKKER et al., 2010; CANALE & HENRY, 2010; DE JONG et al., 2010). Secondary forest populations and the agricultural area populations of *Drosophila* have the shortest development time and the longest in grassland populations, and the forest edge populations were intermediate (VAN DER LINDE & SEVENSTER, 2006). In our previous studies we noticed the same adult size for *in situ Drosophila melanogaster* collected from Bucovăţ forest (CHELU et al., 2008). Geographical variation in traits related to fitness is often the results of adaptive evolution. Stress resistance traits in *Drosophila* show clinal variation, suggesting that selection affects resistance traits either directly or indirectly (SISODIA & SINGH, 2010).

CONCLUSIONS

Phenotypic variability is low in our populations of *Drosophila melanogaster* collected from different ecosystems subjected to abiotic stress. The average of development time in natural populations of *Drosophila melanogaster* was 10.50±0.26 days (at 25 ˚C). We emphasized in this study that *Drosophila melanogaster* Peşteana population had the best motility in larval stage (3.90±0.14), a low mortality in pupa stage (1.50±0.35) and proved to be the most prolific population. Mortality was significantly lower in natural populations compared with the wild type, Oregon, which demonstrate the ability of *Drosophila* to adapt at the environment conditions.

ACKNOLEDGEMENTS

This work was supported by POSDRU/6/1.5/S/21 and 52158/2008 PNII grants.

REFERENCES

BAKKER J., VAN RIJSWIKJ M., WEISSING F., BIJLSMA R. 2010. *Consequences of fragmentation for the ability to adapt to novel environment in experimental Drosophila metapopulations.* Conservation Genetics. Springer, Dordrecht. **11**(2): 435-448.

BIJLSMA R. & LOESCHCKE V. 1997. *Environmental stress, adaptation and evolution*. Basel. Birkhäuser Verlag: 11-15**.**

BUDNIK M. & BRNCIC D. 1983. *Preadult competition between colonizing population of Drosophila subobscura and established populations of Drosophila simulans in Chile*. Oecologia. Springer Verlag, Berlin. **58**: 137-140.

CANALE C. I. & HENRY P. Y. 2010. *Adaptive phenotypic plasticity and resilience of vertebrates to increasing climatic unpredictability*. Climate Research. Inter Research, Oldendorf. **43**: 135-147.

CHELU CRISTINA, POPESCU CRISTINA, BUTNARU GALLIA. 2008. *Essay to point out Drosophila melanogaster ecodiversity by RAPD markers*. Studia Universitatis Vasile Goldis. Arad. **18**(2): 83-87.

DE JONG M. A., KESBEKE F. M., BRAKEFIELD P. M., ZWAAN B. J. 2010. *Geographic variation in thermal plasticity of life history and wing pattern in Bicyclus anynana.* Climate Research. The Netherlands, Leiden. **43**: 91-102.

FOGLEMAN J. C. & WALLACE B. 1980. *Temperature-dependent development and competitive ability of three species of D. affinis group*. American Middle Naturalist. Jstor. **104**(2): 341-351.

HOFFMANN A. A. & PARSONS P. A. 1991. *Evolutionary genetics and environmental stress.* Oxford University Press. **9**. 283 pp.

HOFFMANN A. A. & PARSONS P. A. 1997. *Extreme environmental change and evolution.* Cambridge University Press. 259 pp.

HOFFMANN A. A. & HERCUS M. J. 2000. *Environmental stress as an evolutionary force*. Biosciences. BioOne, Washington. **50**: 217-226.

KOROL A. B. 1999. *Evolutionary Theory and Processes: Modern Perspectives.* The Netherlands. Wasser, Dordrecht: 31-53.

LOESCHCKE V., SORENSEN J. G., KRISTENSEN T. N. 2004. *Ecologically relevant stress resistance: from microarrays and quantitative trait loci to candidate genes - A research plan and preliminary results using Drosophila as a model organism and climatic and genetic stress as model stresses*. Biosciences. Indian Academy of Sciences. India. **29**: 503-511.

MALMENDAL A., OVERGAARD J., BUNDY J. G., SORENSEN J. G., NIELSEN N. C., LOESCHCKE V., HOLMSTRUP M. 2006. *Metabolomic profiling of heat stress: hardening and recovery of homeostasis in Drosophila*. American Journal of Physiology. Physiological Society. 291: 205-212.

RICCI M. & BUDNIK M. 1984. *Influence of temperature, density and interspecific competition on the preadult development of Chilean populations of Drosophila subobscura and Drosophila immigrans.* Brazilian Journal of Genetics. Mendeley. **7**: 255-264.

RUBIN L. 1990. *The Rubin Lab*. Berkeley.California. 185 pp.

SAITOU N. & NEI M. 1987. *The neighbor - joining method: A new method for reconstructing phylognetic trees.* Molecular Biology and Evolution. Oxford University Press. **4**: 406-425.

SISODIA S. S. & SINGH N. D. 2010. *Resistance to environmental stress in Drosophila ananassae: latitudinal variation and adaptation among populations*. Journal of Evolutionary Biology. PubMed. **23**: 1979-1988.

SORENSEN J. G., KRISTENSEN T. N., LOESCHCKE V. 2003. *The evolutionary and ecological role of heat shock proteins.* Ecology Letters. Blackwell Science Ltd. London. **6**: 1025-1037.

VAN DER LINDE K. & SEVENSTER J. G. 2006. *Local adaptation of development time and starvation resistance in eight Drosophila specie of Philippines*. Biological Journal of Linnean Society. Wiley Blackwell, London. **87**: 115-125.

Chelu Cristina, Corneanu Mihaela, Butnaru Gallia
University of Agricultural Sciences and Veterinary Medicine of Banat,
Calea Aradului 119, 300364-Timişoara,
E-mail: cristina.chelu@uex-usamvbt.org
E-mail: micorneanu@yahoo.com

Received: March 30, 2011
Accepted: August 3, 2011