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HEAT SHOCK RESPONSE IN *DROSOPHILA MELANOGASTER* NATURAL POPULATIONS

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ABSTRACT
This study was focused on the response to heat shock of some *Drosophila melanogaster* natural populations collected from different Romanian ecosystems affected by the presence of stressors like: high salinity contained in soils, natural radioactivity, intensive mining activity or drought and aridity. A short heat shock applied to *Drosophila melanogaster* natural populations does not affect lifespan, but the mortality increased in case of *Drosophila melanogaster Socodor* and *Bucovăţ* populations. We did not detect any overexpression for some genes involved in thermal stress as follows: *Hsp 70*, *Map 205*, *Cdc2* and *Dp 53*, respectively *Adh* as reference gene.
KEYWORDS: *Drosophila melanogaster*, populations, heat shock, Hsp 70

INTRODUCTION
Climate change, exerting thermal stress, and habitat destruction and fragmentation, resulting in genetic drift and inbreeding, are amongst the most disturbing human activities that endanger global biodiversity (Joubert and Bijlsma, 2010). the combined effects of habitat destruction and environmental stresses such as pollution, global warming and the introduction of exotic species, causing e.g. increased competition or the spread of novel diseases, are expected to increase extinction rates even more in the near future (Reed et al. 2002, Kristensen et al. 2003, Thomas et al. 2004, Hoffmeister et al. 2005, Root & Schneider 2006). Climate change has had a significant impact on species and populations in the last 30 to 40 yr (IPCC 2007). Consequences of climate change for natural populations include changes in the distributional range of species, shifts in phenology, changes in community structure and habitat loss (Walther et al. 2002, Mawdsley et al. 2009, Chown et al. 2010).To survive, individuals in these populations have to be able to adapt to changing and stressful environmental conditions (Hendry et al. 2008). The ability to cope with changing environmental conditions will depend on the amount of genetic variation in the population and the physiological sensitivity of individuals to these environmental changes (Deutsch et al. 2008, Kellermann et al. 2009, Chown et al. 2010). In other words, 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, this Special, de Jong et al. 2010, this Special) and the amount of genetic variation for various fitness traits that is present in the population for evolutionary adaptation to new conditions (Kellermann et al. 2009, Willi & Hoffmann 2009, Bakker et al. 2010).

The heat shock response is a programmed change in gene expression carried out by cells in response to environmental stress, such as heat. This response is universal and is characterized by the synthesis of a small group of conserved protein chaperones. In *Drosophila melanogaster* the Hsp70 chaperone dominates the profile of protein synthesis during the heat-shock response (Wei et al., 2006).

In the present study, we investigated the response of *Drosophila melanogaster* populations to thermal stress.

MATERIALS AND METHODS
***Drosophila melanogaster* populations.**

In our study we used 5 populations of *Drosophila melanogaster* which were collected from different areas of Romania, including polluted zones as follows: *Socodor* (solonchaks and steppe vegetation, plain area), *Turceni* (submontain hilly area, mines activity), *Bucovăţ* (forest, natural radioactivity), *Giubega* and *Moţăţei* (sand dunes and arid zones, plain area), and as control we used wild type, Oregon. The name of our population becomes 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. We were not able to perform immediately the analyses and for this reason we initiated an experiment using heat shock treatment in order to check gene expression as response to increased temperature.

This experiment was conducted in two repetitions at 25 ˚C using a corn-meal, yeast and sugar medium. We used adult individuals, 1-4 days old, sex-ratio 1:1. Heat shock was done in a water bath, at 37 ˚C during 30 min and immediately 30 larvae were collected from each vial for DNA extraction. Observations were made for 23 days until the last individuals hatched out.

DNA extraction

We chose randomly 30 larvae from each populations and we isolated DNA by rapid and small isolation method after Steller protocol (cited by Rubin, 1990).

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The concentration of extracted DNA was measured at nanodrop and the isolated DNA was diluted at 2 µg/µl.

PCR analysis

In order to see gene expression among our natural populations of *Drosophila melanogaster* we used PCR technique using primers as follows: *Adh* forward 5'CCA-AAC-GGG-GTA-GCT-GTG-AT-3' and reverse 5'ATG-TGC-CAG-TAC-CAA-TGC-AA3', *Hsp70* foward 5'AGCCGTGCCAGGTTTG3' and reverse 5'CGTTCGCCCTCATACA3'; *Map*205 Fw 5'GGGCGAAGGTGTGATGTCTA3' and reverse 5'AACACTGAGCAGGATCCATG3',*Cdc*2 5'ATCGACAAGAGTGGCCTCAT3' and *Dp53* 5'GCTCTTTTCACCCATCTACAG3' and reverse 5′-GTCTCATGGAAGCCAG-3′. PCR mixture was performed in a 25 µl final volum, containing the following components: 1µg/µl DNA, 5 unit of Taq DNA Polymerase (Fermentas), Dream Taq Buffer (Fermentas) 1X, 25 mM $MgCl_2$ (Fermentas), 10 mM dNTPs (Fermentas), 10 and reverse µM primer and H_2O distilled water until final volum. PCR reaction were run in a DNA Thermocyler (Biorad) using the next program: 3 min denaturation at 94°C, followed by 35 cycles of 30 sec at 94°C, 30 sec at 55°C, extension was done at 72°C for 30 sec and final extension at 72°C for 7 min. The PCR products were migrated in agarose gel (1.2%) by electrophoresis in TBE buffer (1X), 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.

RESULTS AND DISCUSSIONS

***Drosophila melanogaster* populations life cycle**

In figure 1 is shown the life cycle for *Drosophila melanogaster* populations subjected to heat shock (37 ºC) comparative with the control (25 ºC). Lifespan average for our populations taken in this study was 11.83±0.00 days in both cases, control (C) and also variants subjected to heat shock (HS) in larvar stage of development. Life cycle was 11 days for the following populations: *Drosophila melanogaster Socodor* C and both *Drosophila melanogaster Bucovăţ* C and HS. Adult stage occurs after 12 days of development in case of the other populations, excepting *Drosophila melanogaster Giubega* which becomes from southern areas of Romania and the metamorphosis took 13 days when we compared with wild type, Oregon with 12 days development in both cases (C and HS).

We also observed larva motility in the third stage of development (Table 1). The population with the best motility was *Drosophila melanogaster Socodor* population (HS) followed by *Drosophila melanogaster Bucovăţ*, collected from Bucovăţ forest (Dolj County) which is characterized by the presence of natural radioactivity. The lowest motility was observed in *Drosophila melanogaster Turceni* C (2.25±0.39 cm) and *Drosophila melanogaster Socodor* C (2.75±0.18). Generally the motility of larvae in the third stage of development vas medium, slightly low in *Drosophila melanogaster Turceni*, this population becoming from an area with intensive mining activity. We also notice that heat shock leads to larval crowding.

It is known that pupa stage development is a sensible period of insects metamorphosis becose pupa is immovable and can be vulnerable in stress conditions. In our experiment pupa appeared in the 7th day of the life cycle in all of natural populations used in this study. After all individuals emerged we counted the dead pupae in all vials (C and HS). The highest level of mortality was obtained for wild type, Oregon (66.00±22.34 for C variant and 52.00±4.26 for HS variant). High rates of mortality were seen in *Drosophila melanogaster Socodor* C (30.00±4.26) and *Drosophila melanogaster Bucovăţ* C (21.00±9.93) and HS (34.50±22.34). The first one was collected from places with high salinity in soils and the second one, Bucovăţ, from forest. The lowest mortality was determinated for 2 populations, *Drosophila melanogaster Giubega* and *Drosophila melanogaster Turceni* (Table 2) becoming from areas characterized by drought and high temperature in case of Giubega and respectivelly zones with intensive exploatation of coal, from this process is resulting energy used by the peoples but olso toxic emissions for humans and animals.

It is known that *Drosophila melanogaster* is a small insect but with a high capacity of reproduction, a female can lay a few hundred of eggs during her life. In this experiment we let the females to deposit eggs during 3 days and then we hached them out. We wanted to see if the capacity of reproduction can be affected by heat shock (Table 3). In this context we obtained the following results: in control variants, the most prolific population has proved to be *Drosophila melanogaster Bucovăţ* (262.00±15.60 individuals), followed closely by *Drosophila melanogaster Moţăţei* (257.50±15.25) and *Drosophila melanogaster Giubega* (219.00±0.71). Lower levels were seen in *Drosophila melanogaster Socodor* (115.00±78.00) and *Drosophila melanogaster Turceni* (91.50±43.63) in comparation with wild type, Oregon (153.00±8.51). Regarding the variants subjected to heat shock, there was not observed a semnificative decrease of prolificacy in our populations collected from different ecosystems, only in Oregon standard type the variant treated with heat shock the livestock was singnificantly lower (62.50±11.7) when we compared with control (153.00±8.51). In case of the populations originating from southern areas of Romania, affected by drought and higher temperatures then other places of our country, prolificacy was higher in control variants by comparison with that ones which were subjected to heat shock at 37 ºC, also in Bucovăţ forest located in the south part characterized by forest and increased humidity.

Tabele 1. Larva motility (cm) in *Drosophila melanogaster* natural populations.

Statistical parameters	Oregon C	Oregon HS	Socodor C	Socodor HS	Turceni C	Turceni HS	Bucovăț C	Bucovăț HS	Giubega C	Giubega HS	Moțăței C	Moțăței HS
$x \pm s_x$	4.45±1.03	3.25±0.04	2.75±0.18	5.95±0.18	2.25±0.39	3.00±0.07	5.25±0.25	4.45±0.32	3.15±0.18	4.75±0.74	4.15±0.11	4.65±0.74
s^2	2.10	0.00	0.06	0.06	0.30	0.01	0.12	0.20	0.06	1.10	0.02	1.10
s%	32.58	1.54	9.09	4.20	24.44	3.33	6.67	10.11	7.94	22.11	3.61	22.58
s	1.45	0.05	0.25	0.25	0.55	0.10	0.35	0.45	0.25	1.05	0.15	1.05

Tabele 2. Pupa mortality in *Drosophila melanogaster* natural populations.

Statistical parameters	Oregon C	Oregon HS	Socodor C	Socodor HS	Turceni C	Turceni HS	Bucovăț C	Bucovăț HS	Giubega C	Giubega HS	Moțăței C	Moțăței HS
$x \pm s_x$	66.00±7.80	52.00±4.26	30.00±2.84	11.50±6.03	5.00±2.84	8.00±1.42	21.00±9.93	34.50±22.34	4.00±0.00	5.50±1.06	13.00±1.42	13.00±1.14
s^2	121.00	36.00	16.00	72.25	16.00	4.00	196.00	992.25	0.00	2.25	4.00	4.00
s%	16.67	11.54	13.33	73.91	80.00	25.00	66.67	91.30	0.00	27.27	15.38	15.38
s	11.00	6.00	4.00	8.50	4.00	2.00	14.00	31.50	0.00	1.50	2.00	2.00

Table 3. Livestock (average of 2 repetitii) in *D. melanogaster* natural populations.

Prolificacy	Oregon C	Oregon HS	Socodor C	Socodor HS	Turceni C	Turceni HS	Bucovăț C	Bucovăț HS	Giubega C	Giubega HS	Moțăței C	Moțăței HS
$x \pm s_x$	153±8.51	62.5±±11.7	115±7.80	284±29.79	91.5±43.62	102.5±32.4	262.0±15.6	237±39.01	219±0.71	181.5±39.36	257.5±15.25	229±4.26
s^2	144.00	272.25	121.00	1764.00	3782.25	2352.25	484.00	3025.00	1.00	3080.25	462.25	36.00
s%	7.84	26.40	9.57	14.79	67.21	47.32	8.40	23.21	0.46	30.58	8.35	2.62
s	12.00	16.50	11.00	42.00	61.50	48.50	22.00	55.00	1.00	55.50	21.50	6.00

Table 4. Sex-ratio (♀: ♂) in *Drosophila melanogaster* natural populations.

Sex-ratio ♀:♂	Oregon C	Oregon HS	Socodor C	Socodor HS	Turceni C	Turceni HS	Bucovăț C	Bucovăț HS	Giubega C	Giubega HS	Moțăței C	Moțăței HS
	0.55: 0.45	0.55:0.45	0.56:0.44	0.53:0.47	0.48:0.52	0.57:0.43	0.52:0.48	0.55:0.45	0.47:0.53	0.47:0.53	0.48:0.52	0.52:0.48

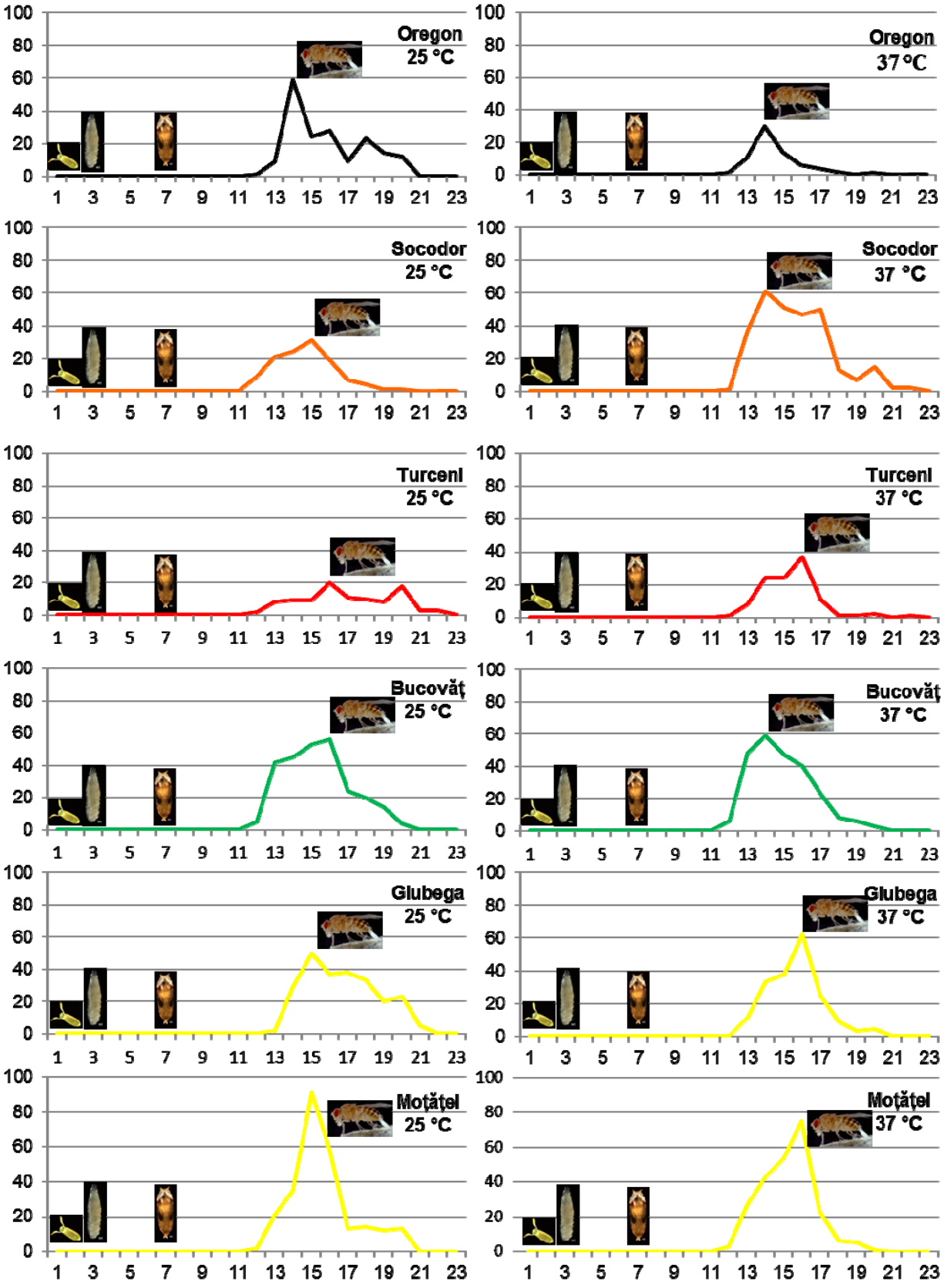

Fig. 1. Life cycle in *Drosophila melanogaster* natural populations in standard laboratory conditions at 25 ºC. The left part shows control variants and the wright part shows variants subjected to heat shock (30 min at 37 ºC).

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In case of 2 populations: *Drosophila melanogaster Socodor* and *Drosophila melanogaster Turceni* we determinated the best prolificacy when were subjected to heat shock and compared with control variants.

Regarding sex ratio, this is quite well-balanced, easily in favor of females in the case of the following populations: *Drosophila melanogaster Socodor* (C and HS), *Turceni* (HS), *Bucovăţ* (C and HS), *Moţăţei* (HS) and respectivelly Oregon (C and HS). The populations were the males dominated were: *Drosophila melanogaster Turceni* (C), *Giubega* (C and HS) and *Moţăţei* (C), but the differences were small and insignificant (Table 4).

PCR results

It is known already that the presence of stress conditions induce gene expression in high levels. This kind of genes involved in response to stress were checked in this study as follows: *Hsp 70*, the most important family genes which is responsible for producing heat shock proteins in case of increased temperatures, also *Map 205* and *Cdc 2*, two genes involved in microtubuli binding process and cell division which can also be affected by stressors. *Dp53* has many roles including response to abiotic stimulus, programmed cell death and determination of adult lifespan (www.flybase.org). We used *Adh* as reference gene, which is the most stable gene from the human and animals genome, so well preserved in *Drosophila melanogaster* genom. Although the synthesis of Hsp70 is nearly undetectable in Drosophila cells at the normal growth temperature of 25°, its expression is rapidly induced at least 1000-fold by raising the temperature to 37° (Velarquez et al.,1983).

In figure 2 we showed the results regarding gene expression in *Drosophila melanogaster* natural populations after 30 min heat shock at 37 ºC in water bath. There is no significant difference between control variants and variants subjected to thermal stress. *Hsp 70* gene shows a higher expression level when we compared with *Adh* gene, but there is no difference between control and heat shock variants. *Map 205*, *Cdc 2* and *Dp53* present just a slighty expression level in both variants.

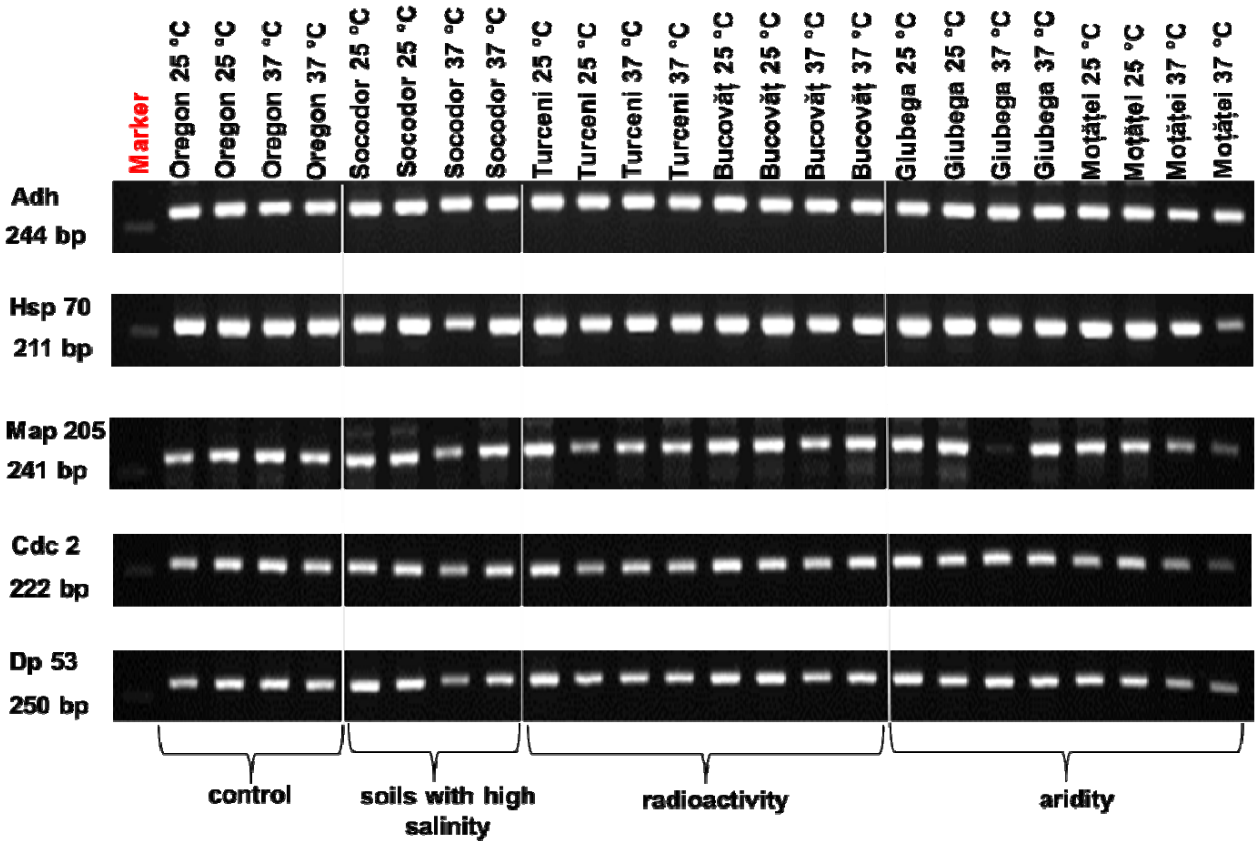

Fig. 2. Gene expression in Drosophila melanogaster populations subjected to thermal stress (30 min at 37 ºC).

Genetic or environmental manipulations to extend lifespan in various organisms have been found to correlate with increases in resistance to environmental stress (Martin et al., 1996; Finkel et al., 2000). *Drosophila* lifespan is increased by overexpression of the antioxidant Cu-Zn superoxide dismutase (SOD) (Orr et al., 1994; Sun et al., 1999; Parkes et al. 1998) or by overexpression of the heat-shock protein (Hsp) gene *Hsp70* (Tatar et al., 1997). The set of Hsp is one of the intracellular defense systems in response to different stressful conditions (Verbeke et al., 2000). Wei et al. (2006) find out that *Hsp70* is essential to survive a severe heat shock, but is not required to survive a milder heat shock, indicating that a significant degree of thermotolerance remains in the absence of *Hsp 70*. HSPs function as molecular chaperones to enhance protein folding, prevent protein denaturation and aggregation, and facilitate proteolysis of damaged proteins. A decrease in the response of HSPs to stress occurs during aging (Rattan et al., 1995). Deterioration of the cell's capacity to produce active HSPs could lead to the accumulation of damaged proteins or lipofuscin (Verbeke et al., 2000; Rattan, 1995; Terman et al., 1998). HSPs could prevent age-associated protein damage and aggregation (Rogalla et al., 1991; Stromer et al., 2003; Haslbeck et al., 1999).

CONCLUSIONS

In this study we shown that lifespan of *Drosophila melanogaster* natural populations is not affected by thermal stress applied for 30 min at 37 ºC. Larvae present a good mobility and the pupa mortality wasn't incresed by thermal stress in our populations, excepting *Drosophila melanogaster Socodor* and *Bucovăţ*, respectivelly standard type, Oregon with a significant mortality, which demonstrate that natural populations of *Drosophila melanogaster* can cope better with the unfavorable environmental coditions.

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