Journal of Applied Biological Sciences 6 (1): 45-49, 2012
ISSN: 1307-1130, E-ISSN: 2146-0108, www.nobel.gen.tr

The Effects of Heat Shock on the Longevity in Some Strains of *Drosophila melanogaster* (Diptera: Drosophilidae)

Arif AYAR[1] Handan UYSAL[1*] Deniz ALTUN[2] Hakan AŞKIN[3]

[1] Department of Biology, Faculty of Science, Atatürk University, Erzurum, 25240, TURKEY

[2] Department of Biology, Faculty of Art and Science, Erzincan University, Erzincan, 24100, TURKEY

[3] Department of Molecular Biology and Genetics, Atatürk University, 25240, TURKEY

[*]Corresponding Author
e-mail: hauysal@atauni.edu.tr

Received : October 15, 2011
Accepted : November 21, 2011

Abstract

It is known that there is a relationship between stress factors and longevity and many stress factors are effective on longevity and aging. One of the stress factors is temperature. Global warming, whose effects keep rising nowadays and better understood by the people, and climate changes depending on global warming are strengthening the effects of this stress factor. In this study, the effects of heat shock on the longevity of *Drosophila melanogaster* were analyzed. The flies used in the experiments were Oregon R wild type and *Vestigial* mutant type of *D. melanogaster*. For this study, a 39°C heat shock was applied to the experimental groups at different durations (1, 2 and 3 hours). According to our results, it was observed that the mean female and male populations life span of the Oregon R wild type and *Vestigial* mutant type of *D. melanogaster* was reduced depending on the increase in the duration on the experimental groups ($p<0.05$ and $p<0.001$).

Keywords: *Drosophila melanogaster*; Global warming; Heat shock; Longevity

INTRODUCTION

Aging, the mechanism of which we still have unknown points about, can be defined as "all of the events in total which are arranged by the genetic code and cause the organism to die due to structural and functional changes" [1]. It was first mentioned by Comfort (1966) [2] that the key to increasing longevity was to understand the formation mechanism of aging and the problems that lie underneath this mechanism. It is known that besides temperature, stress factors are also important factors affecting the longevity of living things [3-5]. Global warming, the effects of which have recently started to be felt more along with the global climate changes due to global warming have brought forward the stress conditions that might arise as a result of heat shocks [6, 7].

Today, one of the most important ecological issues of mankind is "Global Warming and Climate Change" [8]. It is unavoidable that global warming will affect the insects besides all living creatures on the world. It is expected that the changes in temperature, humidity and CO2, as a result of global warming will also affect the insects [9, 10]. Insects are cold-blooded organisms and the temperatures of their body are approximately at the same temperature as their environment. Therefore, the changes in humidity, CO2 and especially in temperature may influence the insect behaviour, distribution, development, reproduction and longevity [11-13].

The fruit fly *Drosophila melanogaster* is one of the favorite models of Biogerontologists. In this study, the influence of heat shock, one of the stress factors effecting the longevity of the Oregon R wild type and *Vestigial* mutant strains of the *Drosophila melanogaster* was investigated.

MATERIALS AND METHODS

Origin and Maintenance of *Drosophila melanogaster*

The flies used in the experiments were Oregon R wild type (w.t.) and *Vestigial* (vg) mutant strains of *Drosophila melanogaster* Meigen (Diptera; Drosophilidae). These stocks have been maintained for many years in the Laboratory at the Department of Biology of the Atatürk University in Erzurum and were, therefore, highly inbred with little genetic variation. The flies were kept at a constant temperature of 25±1°C on standard medium composed of maize-flour, agar, sucrose, dried yeast and propionic acid (Standard *Drosophila* Medium= SDM) [14]. The flies were kept in darkness, except during the transfer onto a fresh medium (usually half weekly). The humidity of the experimental chamber was 40-60%. The females used in this experiment were virgins.

Experimental Protocol

In this study, the effects of heat shock on longevity were studied separately on the female and male populations. To obtain same-aged flies, adult individuals mated in the culture vials with only SDM and prestocks were prepared. On the average, 100 individuals were collected from Oregon R and *Vestigial* of the female and male flies which were not mated. Virgin flies were obtained from pupa at ±8 hours. Then, 100 individuals were put into one vial for the application (separately applied for female and male flies) and then were placed into the culture vials containing only SDM as 25 by 25. After 3 days, the adult female and male flies were separately exposed to heat shocks of various durations (1, 2 and 3 hours) in a thermal cabin set at 39˚C and all

the vials were kept in the appropriate thermal cabins (25±1˚C). During the experiments the adult flies were transferred to fresh vials every 3 days. The number of individuals were controlled both at the beginning and at the end of every application day, and the dead individuals were registered and then removed from the environment. The application was carried out until the last individual died.

Statistical Analyses

The obtained data were analyzed with SPSS (version 13.0). The mean longevity of the control and experimental groups were compared using the Duncan Test and the Games- Howell Test on the probability levels of 0.05 and 0.001.

RESULTS

The Effects of Heat Shock on the Longevity of the Oregon R Strains (w.t.) of *D. melanogaster*

According to our results, it was observed that the maximum life span of the control group was 76 days for the females and 73 days for the males. In both sexes, the maximum life span of the experimental groups ($G1_{wt}$, $G2_{wt}$ and $G3_{wt}$) was compared with the control group, the maximum life span shortened depending on the application durations. In $G1_{wt}$ (exposed to heat shock for 1h), $G2_{wt}$ (exposed to heat shock for 2h) and $G3_{wt}$ (exposed to heat shock for 3h) experimental groups, the maximum female life span was 73, 70 and 67 days respectively. Hovewer, it was determined that the maximum male life span was 70, 61 and 58 days, respectively (Figure 1.).

The maximum mean female and male life span was 56.47±1.5 and 51.22±1.3 days, respectively. The minimum mean life span was 43.39±1.8 days for the females and 37.39±1.5 days for the males. In both sexes, it was determined that the minimum mean life span was in $G3_{wt}$ which was exposed to heat shock for 3 hours (Table 1.).

When the mean life span of the male and female individuals and control group were compared, it was observed that the longevity of the experimental groups was shorter than the control. As shown in Table 1., except for only one experimental group (in females, C_{wt} -$G1_{wt}$), the difference between the groups in longevity was statistically significant ($p<0.05$ and $p<0.001$). When the mean life span of male and female individuals of Oregon R strains of *D. melanogaster* were compared, it was observed that the females in all of the groups survived longer than the males (Table 1.).

The Effects of Heat Shock on the Longevity of *Vestigial* (vg) Mutant Strains of *D. melanogaster*

As seen in Table 1., it was observed that the maximum life span of the control group was 64 days for the females and 70 days for the males. In both sexes, the maximum life span of the experimental groups ($G1_{vg}$, $G2_{vg}$ and $G3_{vg}$) were compared with the control group, the maximum life span shortened depending on the application durations. In $G1_{vg}$, $G2_{vg}$ and $G3_{vg}$ experimental groups, the maximum female life span was 61, 52 and 49 days, respectively. Hovewer, it was determined that the maximum male life span was 64, 55 and 52 days, respectively (Figure 2.).

The maximum mean female and male life span was 46.09±0.9 and 48.01±1.3 days, respectively. The minimum mean life span was 36.79±1.1 days for the females and 34.51±1.5 days for the males. In both sexes, it was determined that the minimum mean life span was in $G3_{vg}$ which was exposed to heat shock for 3 hours (Table 1.).

When the mean life span of the male and female individuals and the control group were compared, it was observed that in all of the experimental groups the life span was shorter than the control group. As shown in Table 1., except for only one experimental group (in females, $G1_{vg}$ -$G2_{vg}$), the difference between the groups in longevity was statistically significant ($p<0.05$ and $p<0.001$).

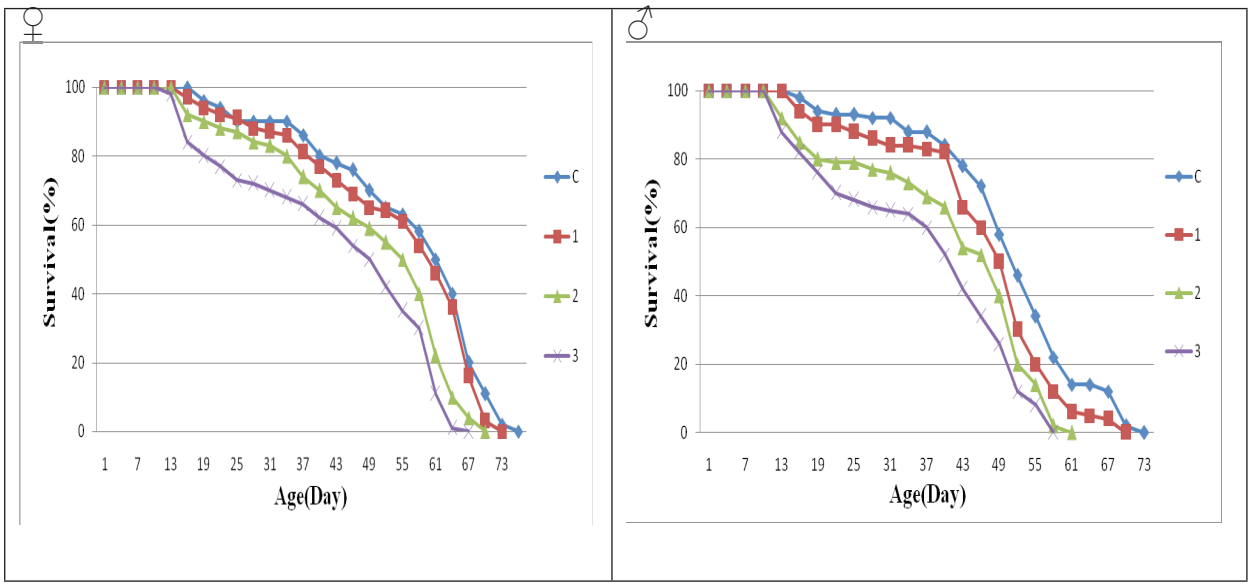

Fig.1. The survival lines of Oregon R wild type of *Drosophila melanogaster* female and male individuals exposed to heat shock at different durations

Table 1. The longevity of Oregon R wild type and *Vestigial* mutant type male and female populations of *Drosophila melanogaster* and the probability levels between groups.

EXPERIMENT GROUPS	GROUP NAME	SEX	N	MAX. LIFE (Days)	MEAN LIFE SPAN (Day)±S.E.	S.D.	PROBABILITY LEVELS BETWEEN GROUPS			
							For Oregon		For *Vestigial*	
							♀	♂	♀	♂
Control	C_{wt}	♂	100	73	51.22±1.3	13.03	C-2* C-3** 1-2* 1-3** 2-3*	C-1* C-2** C-3** 1-2* 1-3** 2-3*	C-1* C-2** C-3** 1-3** 2-3*	C-1* C-2** C-3** 1-2* 1-3** 2-3*
		♀	100	76	56.47±1.5	15.55				
	C_{vg}	♂	100	70	48.01±1.3	13.56				
		♀	100	64	46.09±0.9	9.43				
Application of heat shock (1 hour)	1_{wt}	♂	100	70	47.02±1.3	13.34				
		♀	100	73	54.40±1.6	16.27				
	1_{vg}	♂	100	64	43.90±1.4	14.26				
		♀	100	61	42.70±1.4	14.52				
Application of heat shock (2 hours)	2_{wt}	♂	100	61	41.74±1.5	15.16				
		♀	100	70	49.36±1.6	16.24				
	2_{vg}	♂	100	55	39.13±1.3	13.39				
		♀	100	52	40.36±1.2	12.60				
Application of heat shock (3 hours)	3_{wt}	♂	100	58	37.39±1.5	15.41				
		♀	100	67	43.39±1.8	18.85				
	3_{vg}	♂	100	52	34.51±1.5	15,06				
		♀	100	49	36.79±1.1	11.41				

Max.: Maximum, N: Total number of individuals, S.E.: Standart Error, S.D.: Standard deviation, wt: Oregon R, Vg: *Vestigial*, *: The mean difference is significant at the 0.05 level. **: The mean difference is significant at the 0.05 and 0.001 level.

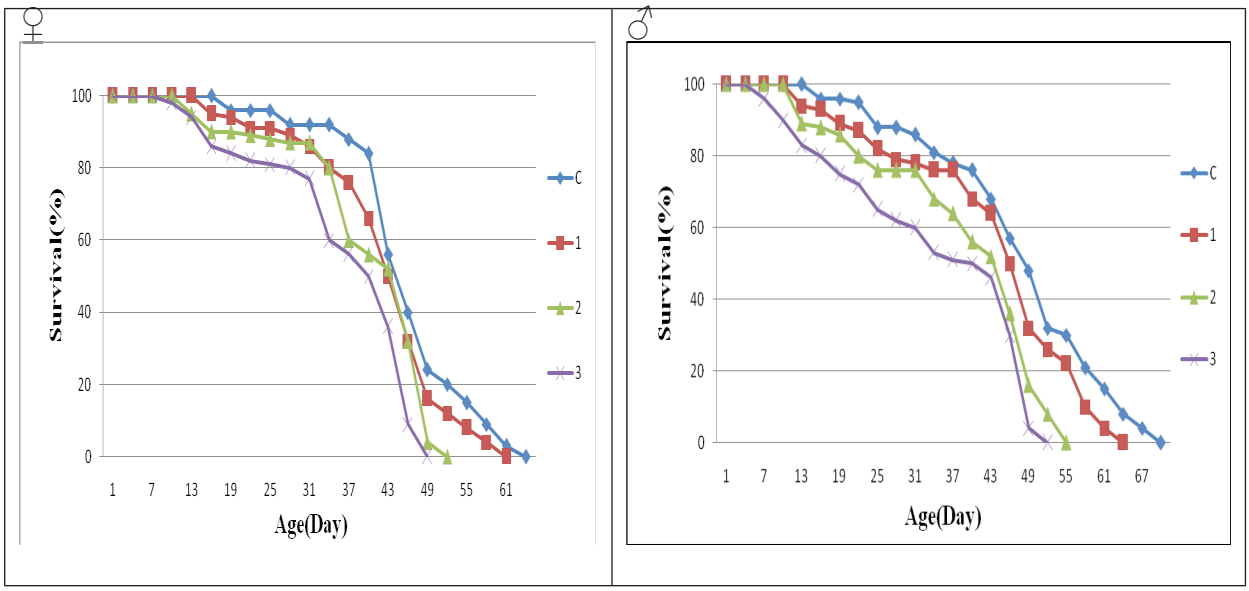

Fig.2. The survival lines of *Vestigial* mutant type of *Drosophila melanogaster* female and male individuals exposed to heat shock at different durations

DISCUSSION

The relationship between the longevity of *D. melanogaster* and temperature was first researched by Loeb and Northrop (1916) [15]. As a result of these studies, it was determined that there is a certain temperature coefficient for the life span and that there is an inverse proportion between longevity and temperature. In another study, the relationship between the applications of heat shock at 37°C at varying daily and weekly periods and longevity was analyzed. As a result, the shortest longevity was determined in the group on which heat shock was applied the most [3]. Pearl and Miner (1935) [16], has stated that aging depends on temperature just like chemical reactions. The longer longevity of *Drosophila* at high temperatures is simply thought to be the result of *Drosophila*'s activity and high metabolism rate at these temperatures [17], have stated that the optimum temperature interval for male and female *Drosophila* individuals is between 16-29°C and that suddenly more deaths are observed below 12°C and above 32.5°C. The results that we have found also support this.

In many studies, some stress factors (i.e: hypergravity, heat or cold shock) when at a low rate and in a short time are applied to *D. melanogaster*, it shows an hormetic effect, but when intensity and time of application were increased, it was observed that there were harmfull effects [18-20]. These results were similiar to ours.

An important part of the cellular response to heat stress is constituted by a group of genes coding for heat shock proteins (HSPs) or stress proteins because their expression can be induced by high temperatures and a whole range of other stress factors [21]. HSPs constitute an inducible part of molecular chaperones that play important roles in transport, folding, unfolding, assembly and disassembly of multistructured protein complexes, signal pathways, degradation of misfolded or aggregated proteins, and the activation of enzymes and receptors [22]. The heat shock genes are upregulated after exposure to stressful, potentially damaging conditions and provide the organism with a temporary enhanced tolerance to stress [23].

The HSPs that were first discovered in *Drosophila* are synthesized in all living things from mammals to bacteria. These proteins are synthesized under stress conditions and surround the other proteins that have important functions for the cell preventing their fractionation or cytolysis. In the study performed on *D. melanogaster* populations, the production of HSP22 was stopped and its effect on longevity was analyzed. As a result, a 40% decrease in the longevity in comparison to the control group was observed in the absence of HSP22 with the heat shock application [24]. In another study, the effect of heat shock on HSP70 production and survival of *D. melanogaster* populations of different age groups (0-8 days) was analyzed. As a result it was observed that there is an inverse proportion between the age groups and HSP70 production and survival [25, 26]. In order to avoid such an effect in our study, individuals at the same age (3 days) were used for all heat shock applications.

According to Nielsen et al. (2005) [27], the most important effect of lethal temperatures is protein denaturation. During shock, the three dimensional structure is disrupted due to the rupturing of the H bonds of some enzymes in the protein structure and along with the increase of entropy; the enzyme loses its catalytic activity. Consequently some physiological events that will continue throughout the life span may take place wrongfully and as a result longevity may decrease. According to another point of view, deaths that occur as a result of heat shock may be linked to the increase of the salt concentration of the body and the increase of free radicals that are formed due to lipid peroxidation [28].

Since insects are cold blooded (polychlothermal) beings, increase in ambient temperature causes an increase in their metabolic activities, respiration rates and free radical formations causing cellular damage and the decrease in longevity. It means that as temperature which enables life increases, heat production and oxygen usage increases while longevity decreases [29]. Whereas free radicals cellular activities at normal physiological concentrations, they may cause oxidative stress and become toxic at high concentrations. As a result they increase the aging process causing genetic, metabolic and neurodegenerative disorders and even cancer [30, 31]. Strehler (1961) [32] has suggested that aging is dependent upon temperature and has explained the possible mechanisms between aging and temperature with the increase of the high temperature degeneration rate of temperature sensitive structure and functional elements such as cells, proteins and nucleoproteins, the increase of the catabolism and usage rates of various metabolites such as cofactor and catalyst, the increase at high temperatures of the accumulation rate of various toxic materials or the fact that they can't be easily thrown out of the body.

Though natural species are capable of adapting to climate changes in a long process, many plants and animals cannot adapt to rapid climate changes. Our results suggest that, extreme temperature conditions provided by rapid temperature changes, shorten male and female longevity of the Oregon R wild and *Vestigial* mutant strains of *D. melanogaster*. Any other effects of global warming and climate changes on the longevity of species should be investigated by other studies.

REFERENCES

[1] Bozcuk AN. 1981. Genetics of Longevity in *Drosophila*. The specific and hybridised effects of rolled, speia, ebony and eyeless autosomal mutants. Experimental Gerontolology. 16:415-427.

[2] Comfort A. 1966. The prevention of aging in cells. Lancet. 17:1325-1329.

[3] Le Bourg E, Valenti P, Lucchetta P, Payre F. 2001. Effects of mild heat shock at young age on ageing and longevity in *Drosophila melanogaster*. Biogerontology. 2:155-164.

[4] Vermeulen CJ and Bijlsma R. 2004. Characterization of Conditionally Expressed Mutants Affecting Age-Specific Survival in Inbred Lines of *Drosophila melanogaster:* Lethal Conditions and Temperature-Sensitive Periods. Genetics. 167:1241-1248.

[5] Vermeulen CJ and Loeschcke V. 2007. Longevity and the stress response in *Drosophila*. Experimental Gerontology. 42:153-159.

[6] Bradshaw WE and Holzapfel CM. 2001. Genetic shift in photoperiodic response correlated with global warming. The Proceedings of the National Academy of Sciences Online (U.S.A.). 98:14509–14511.

[7] Houghton J. 2005. Global warming. Reports on Progress in Physics. 68:1343-1403.

[8] Sağlam NE, Düzgüneş E, Balık İ. 2008. Global warming and change climate. Journal of Fisheries and Aquatic Science. 25:89-94.

[9] Harrington R, Fleming RA, Woiwod P. 2001. Climate change impacts on insect management and conservation in temperate regions: can they be predicted? Agricultural and Forest Entomology. 3:233-240.
[10] Bale JS, Masters GJ, Hodkinson ID, Awmack C, Bezemer TM. 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. Global Change Biology. 8:1-16.
[11] Ward NL, Masters GJ. 2007. Linking climate change and species invasion: an illustration using insect herbivores. Global Change Biology. 13:1-11.
[12] Ozgen I and Karsavuran Y. 2009. The evoluation of global climate alterations from point of insects view. Journal of Harran University Agricultural Science. 13:51-61.
[13] Robınet C and Roques A. 2010. Direct impacts of recent climate warming on insect populations. Integrative Zoology. 5:132-142.
[14] Bozcuk AN. 1978. The effect of some genotypes on the longevity of adult *Drosophila*. Experimental Gerontology. 13:279-285.
[15] Loeb J and Northrop JH. 1916. Is there a temperature coefficient for the duration of life? The Proceedings of the National Academy of Sciences Online (U.S.A.). 2:456-457.
[16] Pearl R and Miner JR. 1935. Experimental studies on the duration of life XIV: The comparative mortality of certain lower organism. The Quarterly Review of Biology. 10:60-79.
[17] Economus AC and Lints FA. 1986. Developmental temperature and life span in *Drosophila melanogaster*. Gerontology. 32:18-27.
[18] Le Bourg E. 2007. Hormetic effects of repeated exposures to cold at young age on longevity, aging and resistance to heat or cold shocks in *Drosophila melanogaster*. Biogerontology. 8:431- 444.
[19] Gem D and Partridge L. 2008. Stress response, hormesis and aging: "That which does not kill us makes us stronger". Cell Metabolism. 7:200-203.
[20] Ayar A, Uysal H, Altun D. 2009. The effects of cold shock on the longevity in Oregon R wild and *vestigial* mutant of *Drosophila melanogaster* (Diptera: Drosophilidae). Ekoloji. 19:38-44.
[21] Sorensen JG, Kristensen TN, Loeschcke V. 2003. The evolutionary and ecological role of heat shock proteins. Ecology Letters. 6:1025–1037.
[22] Parsell DA and Lindquist S. 1993. The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. Annual Review of Genetics. 27:437–496.
[23] Feder ME and Hofmann GE. 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annual Review of Physiology. 61:243–282.
[24] Morrow G, Battistini S, Zhang P, Tanguay RM. 2004. Decreased lifespan in the absence of expression of the mitochondrial small heat shock protein hsp22 in *Drosophila*. Journal of Biological Chemistry. 42:43382-43385.
[25] Sorensen JG and Loeschcke V. 2002. Natural adaptation to environmental stress via physiological clock-regulation of stress resistance in *Drosophila*. Ecology Letters. 5:16-19.
[26] Morrow GM and Tanguay RM. 2003. Heat shock proteins and ageing in *Drosophila melanogaster*. Seminars in Cell and Developmental Biology. 14:291-299.
[27] Nielsen MM, Overgaard J, Sorensen JG, Holmstrup M, Justesen J, Loeschcke V. 2005. Role of HSF activation for resistance to heat, cold and high-temperature knock-down. Journal of Insect Physiology. 51:1320-1329.
[28] Harman D. 1956. Ageing: A theory based in free radicaland radiation chemistry. Journal of Gerontology. 2:298-300.
[29] Setsini EA, Carlson JC, Allsopp R. 1991. The effects of ambient temperature on life span, lipid peroxidation, superoxide dismutase, and phospolipase A2 activity in *Drosophila melanogaster*. Experimental Gerontology. 26:385-395.
[30] Ames BN, Shigenaga MK, Hagen TM. 1993. Oxidants, antioxidants, and the degenerative diseases of ageing. The Proceedings of the National Academy of Sciences Online (U.S.A.). 90:7915-7922.
[31] Droge W. 2002. Free radicals in physiological control of cell functions. Physiological Reviews. 82:47–95.
[32] Strehler BL. 1961. Studies on the comparative physiology of ageing. II. On the mechanism of temperature life-shortening in *Drosophila melanogaster*. Journal of Gerontology: Medical Science. 16:2-12.