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The Effects of Cold Shock on The Longevity in Oregon R wild and *Vestigial* mutant of *Drosophila melanogaster* (Diptera: *Drosophilidae*)

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Abstract

The affects global warming and climate change promise to be one of the most important environmental problem in the next millenium. It is indicated that an increase in temperature by changes in global climate will not only happen at the same time, but some regions may incur in the sudden cooling.

In this study, the effects of cold 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 -3°C cold 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.

The difference in terms of life span was statistically significant ($p<0.05$ and $p<0.001$) according to the control group.

Keywords: Cold shock, *Drosophila melanogaster*, global warming, longevity.

***Drosophila melanogaster* (Diptera: Drosophilidae)'in Oregon R Yabanıl ve *Vestigial* Mutant Soylarında Soğuk Şokunun Ömür Uzunluğuna Etkileri**

Özet

Küresel ısınma ve iklim değişikliği önümüzdeki yüzyılın en önemli çevre sorunlarından birisi olacaktır. Küresel iklimdeki değişikliklerle sadece sıcaklıkta bir artışın olmayacağı aynı zamanda bazı bölgelerde de ani soğumaların meydana gelebileceği belirtilmektedir.

Bu çalışmada, soğuk şokunun *D. melanogaster*'in Oregon R yabanıl ve *Vestigial* mutant soylarında ömür uzunluğu üzerine etkisi araştırılmıştır. Bu amaçla deney gruplarına farklı sürelerde (1, 2 ve 3 saat) -3°C'de soğuk şoku uygulanmıştır. Kontrol ve deney gruplarından elde edilen verilere göre, hem Oregon R yabanıl hem de *Vestigial* mutant soylarının dişi ve erkek gruplarında artan uygulama sürelerine paralel olarak ortalama ömür uzunlukları kısalmıştır.

Kontrol ve deney grupları arasında ortalama ömür uzunluğu bakımından ortaya çıkan farklılıklar çeşitli düzeylerde ($p<0,05$ veya $p<0,001$) istatistiksel olarak anlamlı bulunmuştur.

Anahtar Kelimeler: *Drosophila melanogaster*, küresel ısınma, ömür uzunluğu, soğuk şoku.

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INTRODUCTION

The use of fossil fuel, industrialization, energy production, disappearance of forests, and other human activities occuring as a result of "global warming and climate change" is threatening the world and is one of the most important environmental problems (Sağlam et al. 2008). Probably, global climate changes will influence the lifestyle and behavior of many animals (Root et al. 2003, Parmesan 2006). In particular, a sudden increase or decrease in temperature, occurance of humidity level changes, metabolism (Çakır and Bozcuk 2000), reproductive capacity, and the dietary habits of insects and depending on the effects of these may affect their life span (Akbulut 2000, Bale et al. 2002, Özgen and Karsavuran 2009).

In this study, the influence of cold 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

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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) (Bozcuk 1976). 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 cold 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 cold shocks of various durations (1, 2 and 3 hours) in a water-bath set at -3°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

In the present study, the changes on the longevity of both strains of *D. melanogaster*, which were exposed to cold shock at different durations, was investigated.

The Effects of Cold 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 cold shock for 1h), $G2_{wt}$ (exposed to cold shock for 2h) and $G3_{wt}$ (exposed to cold shock for 3h) experimental groups, the maximum female life span was 73, 70 and, 64 days respectively. Hovewer, it was determined that the maximum male life span was 70, 67 and 61 days, respectively (Fig. 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 39.16±1.4 days for the females and 39.73±1.2 days for the males. In both sexes, it was determined that the minimum mean life span was in $G3_{wt}$ which was exposed to cold 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 some experimental groups (in females, C_{wt} -$G1_{wt}$; in males, C_{wt} -$G1_{wt}$ and $G1_{wt}$ -$G2_{wt}$), 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, except for the $G3_{wt}$ (Table 1).

The Effects of Cold 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 (G1vg, G2vg and G3vg) were compared with the control group, the maximum life span shortened depending on the application durations. In G1vg, G2vg and G3vg experimental groups, the maximum female life span was 61, 55 and 52 days, respectively. Hovewer, it was determined that the maximum

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				
		♀	100	76	56,47±1,5	15,55	C-2**	C-2*	C-2**	C-2*
	C_{vg}	♂	100	70	48,01±1,3	13,56	C-3**	C-3**	C-3**	C-3**
		♀	100	64	46,09±0,9	9,43				
Application of cold shock (1 hour)	1_{wt}	♂	100	70	48,97±1,3	13,04	1-2*	1-3**	1-2*	1-3**
		♀	100	73	52,90±1,6	16,22	1-3**	2-3*	1-3**	2-3*
	1_{vg}	♂	100	67	45,46±1,2	12,60	2-3*		2-3*	
		♀	100	61	44,68±1,0	10,25				
Application of cold shock (2 hour)	2_{wt}	♂	100	67	44,56±1,2	12,93				
		♀	100	70	45,04±1,5	15,52				
	2_{vg}	♂	100	64	43,24±1,2	12,31				
		♀	100	55	39,16±1,1	11,78				
Application of cold shock (3 hour)	3_{wt}	♂	100	61	39,73±1,2	12,67				
		♀	100	64	39,16±1,4	14,32				
	3_{vg}	♂	100	55	36,85±1,1	11,85				
		♀	100	52	33,82±1,2	12,88				

Max.: Maksimum, 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.

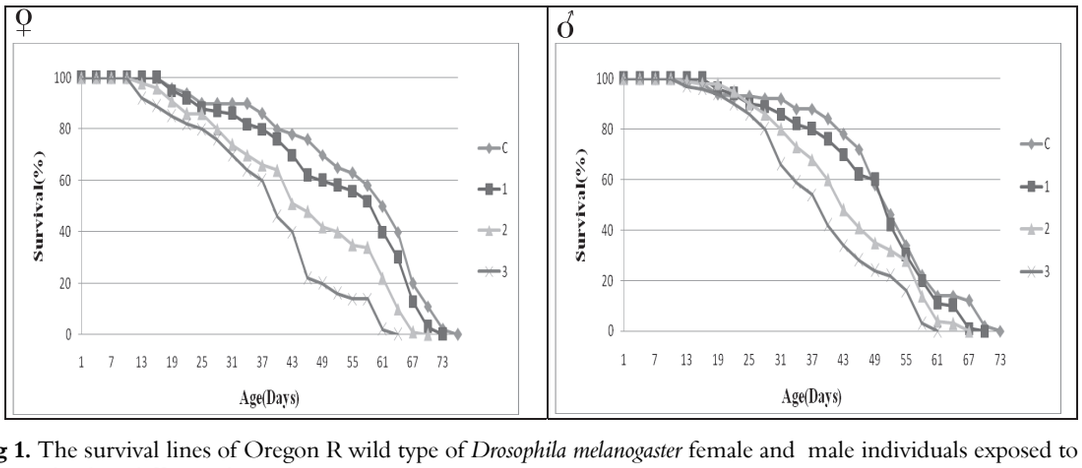

Fig 1. The survival lines of Oregon R wild type of *Drosophila melanogaster* female and male individuals exposed to cold shock at different durations.

male life span was 67, 64 and 55 days, respectively (Fig. 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 33.82±1.2 days for the females and 36.85±1.1 days for the males. In both sexes, it was determined that the minimum mean life span was in G3vg which was exposed to cold shock for 3 hours (Table 1).

When the mean life span of the male and female

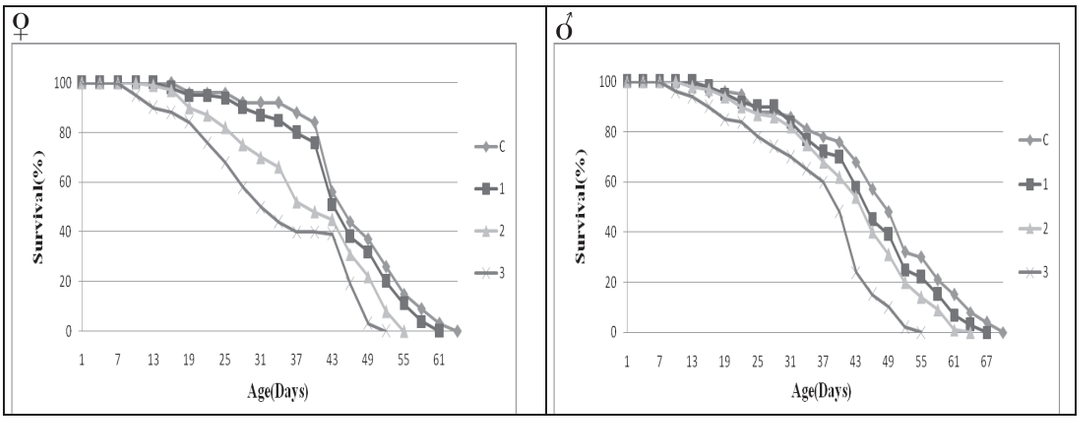

Fig 2. The survival lines of *Vestigial* mutant type of *Drosophila melanogaster* female and male individuals exposed to cold shock at different durations.

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 some experimental groups (in females, Cvg -G1vg; in males, Cvg -G1vg and G1vg -G2vg), 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 *Vestigial* mutant strains of *D. melanogaster* were compared, it was observed that males in all of the groups survived longer than females (Table 1).

DISCUSSION

It is known that there is a relationship between stress factors and longevity and many stress factors have an effect on longevity and aging (Le Bourg et al. 2001, Vermeulen and Bijlsma 2004). 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 increasing the effects of this stress factor (Houghton 2005).

Life span varies in different species, between sexes of the same species, and mutant strains of the same species, of *Drosophila* (Bozcuk 1978, Yeşilada et al. 1994, Carey et al. 1999, Sarıkaya et al. 2006). In addition, the geography and seasonal changes effect the life span, too (Dalgıç 2003).

In our study, the external (environmental) or internal factors that may affect the longevity of the groups were reduced to minimum levels in the application environment. One of these factors is photoperiod and it was determined to be effective during the period of laying egg and emerging from the pupa (Qiu and Hardin 1996), on metabolic velocity (Lanciani et al. 1991) and the longevity (Sheeba et al. 2000) of *D. melanogaster*. Individuals of the control and experimental groups were removed from the incubator only during nutrient exchange and thus the impact of light on longevity was corrected. In our study, only the Standard *Drosophila* Media (SDM) was used instead of different types of food to observe its effect on longevity (David et al. 1975). Maternal age is known to be an important factor on longevity of offspring (Lansing 1952, Sorensen and Loeschcke 2002, Yılmaz et al. 2008). Therefore, we used virgin and even aged individuals in the experiment. Fred and Timothy (1997), in their study, with ten different populations of *Drosophila*, ascertained that longevity increased when metabolic wastes did not exist in the media. So, the negative effect of the waste on the longevity was corrected via frequent renewal of the media.

The above-mentioned internal and external factors that may affect the longevity were fixed during our experiment. Thus, we can tell that, the deviations on the longevity of the flies, under equal conditions identified and in the same genotype, were the result of the effect of the exposure to cold.

Furthermore, Nelson et al. (2006) have expressed that the method of anesthesia changes the results on the applications of cold shock or RCH on D.melanogaster and the CO2 anesthesia gives more accurate results in relationship to the N2 (Anoxia) anesthesia. We did not in any phase of our study use these methods of anesthesia.

Male and female individuals of the *Vestigial*

mutants that were exposed to cold shock for different periods (1, 2 and 3 h) had a shorter life span than the Oregon R wild type male and female individuals (Tables 1).

The general stress response involves the expression of stress proteins, such as chaperones and antioxidative proteins, downregulation of genes involved in energy metabolism and the release of protective substances (Vermeulen and Loeschcke 2007). According to another opinion; adaptative responses of ectothermic organisms to thermal variation typically involve the reorganization of membrane glycerophospholipids (GPLs) to maintain membrane function (Overgaard et al 2008).

It was reported that the low temperature caused *Galleria mellonella* (Wax mouth) larvae to molt more than normal, form tiny larvae, form abnormal pupae and have adults with abnormal wings (Cymborowski 1991). According to Garcia et al. (2001), such abnormalities observed at various development stages of insects after the cold shock was applied, may change their longevity.

Most of the studies about cold stres are based on the understanding of the difference between cold shock and RCH (Rapid cold hardening). Result of these studies imply that cold shock applications have more detrimental effects on longevity than RCH applications (Kelty and Lee 1999, Sorensen and Loeschcke 2002, Sinclair and Roberts 2005, Kelty 2007). In our study, cold shock was applied directly to the experimental groups.

In many studies, some stress factors (i.e: hypergravite, 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 (Le Bourg 2007). These results were similiar to ours.

In earlier studies, direct application of cold shock brought about sudden structural changes on proteins and cell membranes (Czajcka and Lee 1990, Kelty and Lee 1999). Induction of stress proteins such as cryoprotectants may also play a role for the cold shock response in some insects, while the importance of these factors is less clear in the case of *D. melanogaster* (Chen et al. 1987, Joplin et al. 1990, Chen and Denlinger 1992, Kelty and Lee 2001, Nielsen et al. 2005, Qin et al. 2005). Yi et al. (2007) determined that apoptosis or programmed cell death occured with the application of cold shock in *D. melanogaster* but the RCH pre-treatment reduced the amount of apoptosis. Lee et al. (1987) found an increase in the concentration of cryoprotectant after 0°C pre-treatments in the flesh fly (*Sarcophaga crassipalpis*). We applied cold shock as 1, 2 and 3 hour periods in our experiments. Depending on the increase in the duration, degeneration in the structure of the criyoprotectans may have reduced longevity. Furthermore, as a result of the cold shock the amount of cholesterol in the cell membrane was reduce also (Kelty and Lee 2001). According to Shreve et al. (2007), the shortening of longevity may be associated with the decrease in the amount of cholesterol. The authors found that, when the amount of cholesterol in the cell membrane increased, individuals of *D. melanogaster* were more resistant to cold shock and had a longer life. Overgaard et al.(2007) indicated that following RCH and cold shock application, the most significant changes was the amount of sugar and trehalose. As the results show the RCH and cold shock adaptation are claiming to be cryoprotectants.

Cold shock causes insects to lose motor activity in a reversible state known as chill coma (Lee 1991). Although the mechanisms of the chill coma onset are poorly understood, Goller and Esch (1990) suggest that it results from a loss of function of the ion channels necessary for maintaining the membrane potential, leading to voltage equilibration and a loss of muscle cell excitability. Resting potentials in insects are mostly maintained by a Na+/K+ ATPase (Huddart and Wood 1966, Rheuben 1972, Hosler et al. 2000). It is very likely that dysfunction of these pumps depending on duration and amount of cold shock and has negative effects on longevity.

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.

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