

ISSN 2320-7078

JEZS 2014; 2 (2): 163-170 ©
2014 JEZSReceived: 03-02-2014
Accepted: 10-03-2014**Koushik Ponnanna CR***Drosophila Stock Center, Department
Of Studies in Zoology, University of
Mysore, Manasagangothri, Mysore-
570006.*Email: koushik.c.r@gmail.com**Krishna MS***Drosophila Stock Center, Department
Of Studies in Zoology, University of
Mysore, Manasagangothri, Mysore-
570006*Email: drosokrish@gmail.com

Short term sub lethal temperature treatment increases starvation resistance in *D. melanogaster*

Koushik Ponnanna CR and Krishna MS

ABSTRACT

Today's changing environment imposes challenge to the regular homeostasis of organisms, which forces them to face periods of stressful conditions. The changing environmental conditions can evoke a genetic response in organisms which is of a major interest of study in population biology. Influence of short term sub lethal temperature exposure on starvation resistance has been studied in out bred population of *Drosophila melanogaster*. It was noticed that short term sub lethal temperature treated flies significantly mated faster, copulated longer, transferred greater quantity of Accessory proteins to mated females and these females had greater resistance to starvation than untreated flies suggesting that during extreme thermal conditions and food deprivation, mating is highly beneficial for females as it confers resistance towards starvation. This study rules out the idea that mating may impair female fitness as proposed earlier.

Keywords: *Drosophila melanogaster*, starvation resistance, sub lethal temperature, accessory gland proteins

1. Introduction

The homeostasis of organisms has been under constant pressure due to the changing environment which has forced them to face periods of stressful conditions^[11]. The changing environmental conditions can evoke a genetic response in organisms which is of a major interest of study in population biology. Drastic changes caused by environment and human activities impart mechanisms which allow the species to survive and adapt to such changes. Focus on such genetic response can help in understanding the adaptation mechanisms^[1]. Temperature function has been investigated and characterized for extreme conditions and tolerance of cold and heat stress^{[16][18]}. Except that in nature, there is a greater chance for the ectotherms to undergo transient weather conditions that will have direct effect on their body temperature^[20]. Most of the present research focuses on studies carried out by maintaining organisms in the laboratory under fixed rearing temperatures. Not much data is available with regards to short-term exposure to extreme temperatures except in regards with few^[4, 7, 21, 33, 36]. Studies in *D. melanogaster* for chronic temperature exposure have provided a lot of information pertaining to the performance and life history traits^[8, 16]. As observed by Levins,^[22] pre-exposure to a non-lethal high temperature can improve the fitness of flies exposed to heat stress. Fecundity has been shown to increase with temperature upto 25 °C with a marking decrease between 28° to 30°^[9, 19, 24, 30, 32]. Pretreatment has shown improved survival rate for both sexes of *D. buzzatii* with an increased probability of females producing any offspring following the stress.

Negative effects of short term high temperature exposure have been associated with the induction of the heat shock response as a protein quality control system^[34]. Benefits of a short term high temperature treatment are usually not instantaneous, tending to reach a maximum several hours after the treatment^[6], with hardening-induced changes in gene expression that mostly return to its normal values after about 4h. Thus, negative and positive consequences of short term temperature treatment run on a different time course. For instance, a mild heat stress reduces fecundity only during the time of exposure, while benefits of this hardening treatment on heat resistance and longevity can last much longer^[14].

Correspondence:**Krishna MS***Drosophila Stock Center,
Department Of Studies in Zoology,
University of Mysore,
Manasagangothri, Mysore- 570006
Email: drosokrish@gmail.com*

Aim of the present study was to evaluate the influence of short term sub lethal temperature exposure on starvation resistance in *D. melanogaster*. Most of the starvation resistance studies on *D. melanogaster* have been concerned with the relationship between caloric restriction and aging^[31, 28]. Relationship between short term temperature stress and starvation resistance needs to be viewed upon and validated as there is a higher chance in natural environment for the flies to undergo such changes. It was also suggested that starvation resistance is a part of general stress resistance mechanism and understanding the ecological significance and evolution of this apparent relationship has seen little progress in the last decade^[27, 29].

Our present study aims at documenting the variations in the quantity of Accessory protein secretions (Acps) among short term sub lethal temperature treated and untreated flies and its relation to starvation resistance. It also aims at deducing the relation between duration of copulation and quantity of Acps transferred to females.

2.1. Materials and Methods

Experimental outbred population of *D. melanogaster* was established from progenies of fifty isofemale lines collected from domestic localities of Mysore, Karnataka state, India. Stocks were maintained at 22 °C and 70% humidity with a 12L: 12D cycle using standard wheat cream agar medium. Flies emerged in each generation were mixed together and redistributed to new culture bottles (10 males and 10 females per bottle). This procedure was continued for 3 generations to acclimatize the flies to laboratory environment. Embryos were collected from 4th generation by Delcour's procedure and around hundred eggs were seeded into fresh wheat cream agar media bottles. On emergence of adults, unmated males and virgin females were isolated (within 3 hrs of eclosion) and aged for 5-6 days. These flies were used for further experiments.

2.2. Short term sub lethal temperature treatment

Five to six days old unmated male/ female flies were taken in pre heated (37 °C/40 °C) empty glass vials that were plugged with damp cotton soaked in phosphate buffer saline (PBS) to prevent desiccation followed by an exposure to 37 °C for 60 mins 40 °C for 30 mins. These treated flies were then utilised to study mating latency, copulation duration, quantity of accessory gland protein and starvation resistance. To determine the exposure time, four replicates with 10 flies each were exposed to 30 min, 60 min and 90 min. Survival rate was more than 65-70% in groups exposed to 60 min at 37 °C and 30 min at 40 °C. But no flies survived the 90 min exposure period. Hence, throughout the study the treatment was fixed for 60min at 37 °C and 30 min at 40 °C. From here onwards the short term sub lethal temperature exposed flies will be referred as treated flies.

2.3. Analysis of starvation resistance for short term temperature treatment

Five to six days old mated and unmated males and females were obtained from untreated and treated groups. Ten from each group were transferred into vials containing nonnutritive agar (12.4 g agar and 2.4 g p-hydroxybenzoic acid in 23 ml ethanol per litre). The number of days survived by each fly was recorded by observing the vials every 12-24 hours for mortality, until all the flies died. A total of 4 replicates (40 males and 40 females) were observed separately for both mated and unmated flies. The results obtained were subjected to one way ANOVA, followed by Tukey's post hoc test. Kaplan-Meier survival curves were plotted using the data obtained.

2.4. Determination of body weight of the flies.

To weigh the body weight of male and female, 5-6 days old male/female of *D. melanogaster* were taken separately and body weights were measured using a digital balance. A total of thirty replicates were made for each sex. Paired t- test was carried out on the above data.

2.5. Sample preparation of Acps from unmated males

Accessory glands of unmated (etherized) males (untreated and treated male) were individually dissected with insect saline using entomological needles and were immediately fixed in 95% ethanol. The membrane from the fixed glands was removed and the resulted secretion was washed in a mixture of methanol and chloroforms (1:1) and dried at 37 °C in the incubator for 15 min. About 100 µl of buffer (0.625 M Tris HCl) was added to each sample to dissolve the glands and secretions. Quantitative estimation of total Acps was performed using Lowry's protein estimation method for 10 pairs of accessory glands (~50 µl) from each group. One way ANOVA followed by Tukey's post hoc was carried out for the data obtained.

2.6. Sample preparation of Acps from mated males

To obtain mated male, a 5-6 days old virgin female and an unmated untreated and treated males were individually aspirated into Elens-Wattiax mating chamber and observed for 1 hour. Pairs remain unmated within 1 hour were discarded. If mating occurs, mating latency (time between introduction of male and female into a mating chamber until initiation of copulation) and the copulation duration (time between initiations to termination of copulation of each pair) are recorded. Soon after copulation (within 5 minutes), mated males were etherized and Acps were collected and estimated quantitatively as described in section 2.4.

3. Figures and Tables

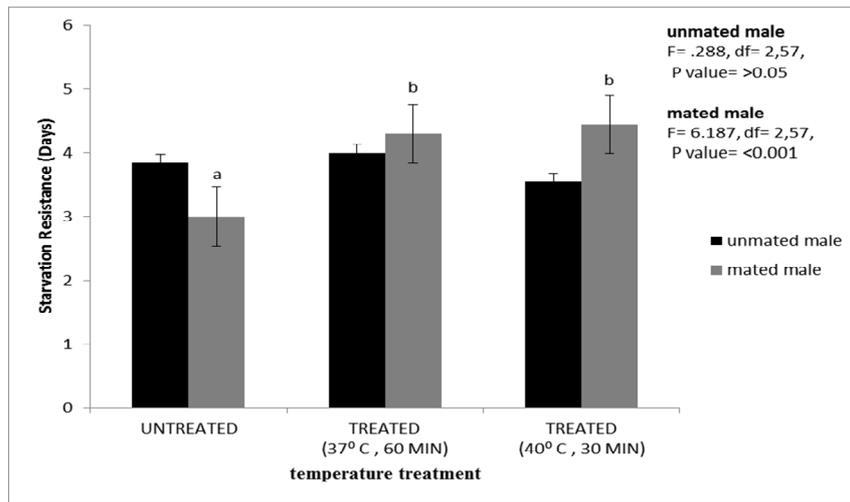


Fig 1: Starvation resistance of mated and unmated males of *D. melanogaster* for both untreated and treated groups. Different letters in the bar graph indicate significance at $p < 0$ by Tukey's post hoc test

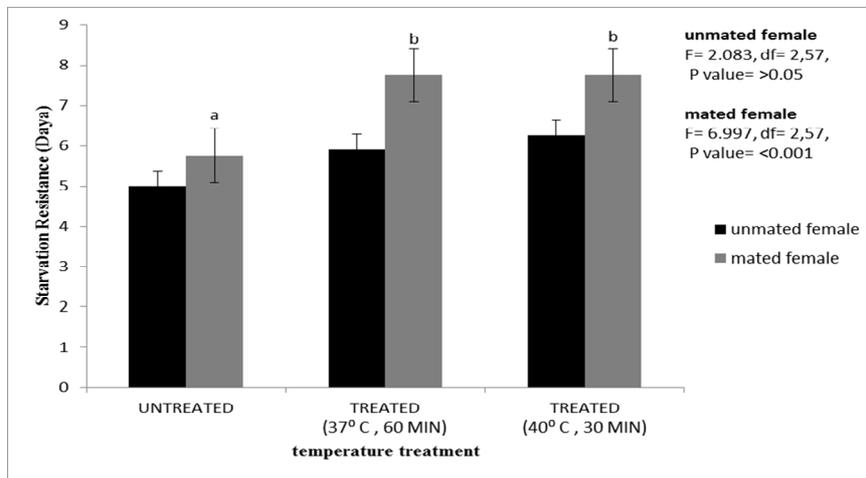


Fig 2: Starvation resistance of mated and unmated females of *D. melanogaster* for both untreated and treated groups. Different letters on bar graph indicate significance at $p < 0.05$ level by Tukey's post hoc test

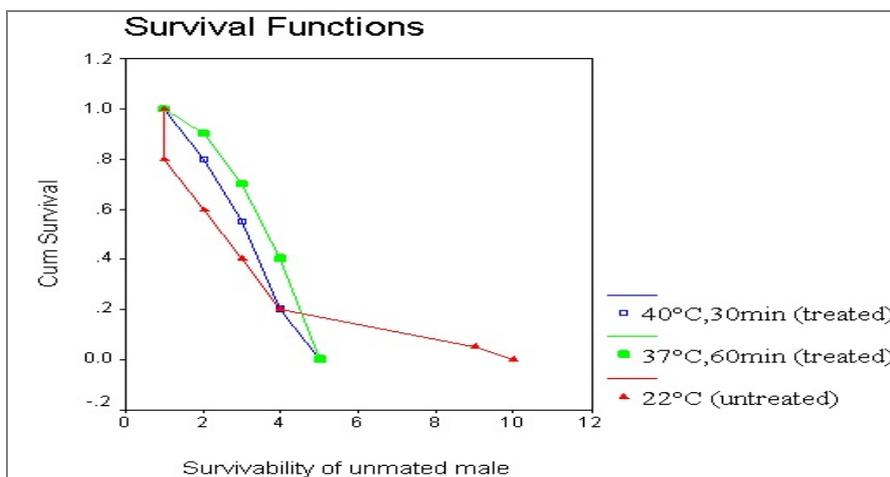


Fig 3: Survival curve of unmated males for untreated and treated group

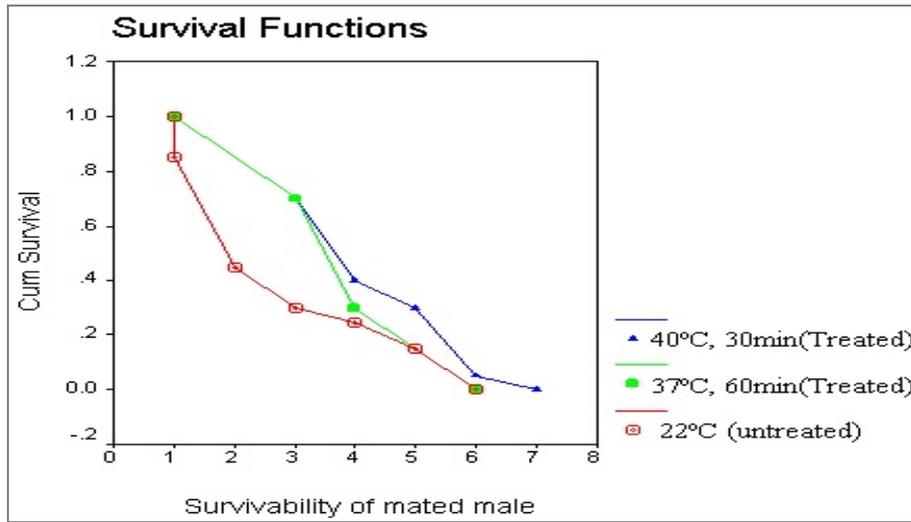


Fig 4: Survival curve of mated males for untreated and treated groups.

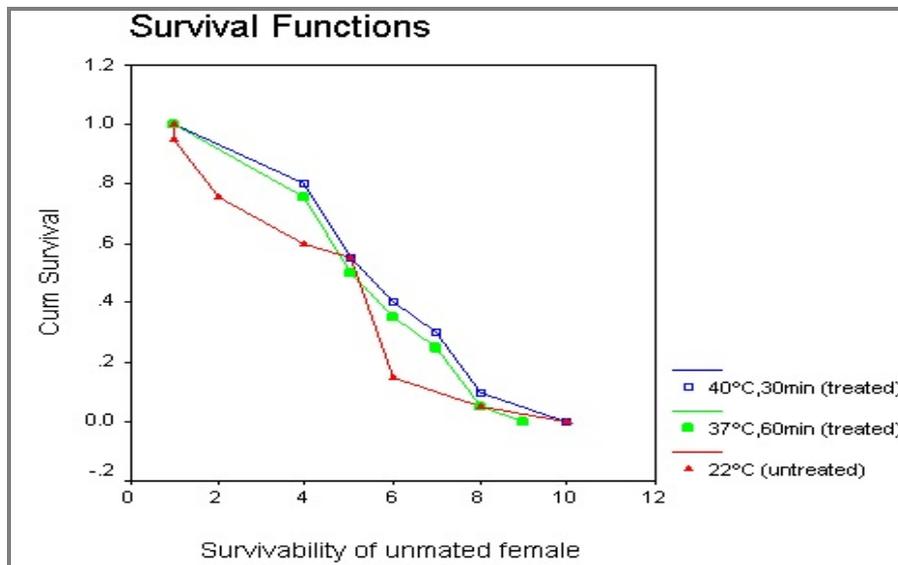


Fig 5: Survival curve of unmated females for untreated and treated groups.

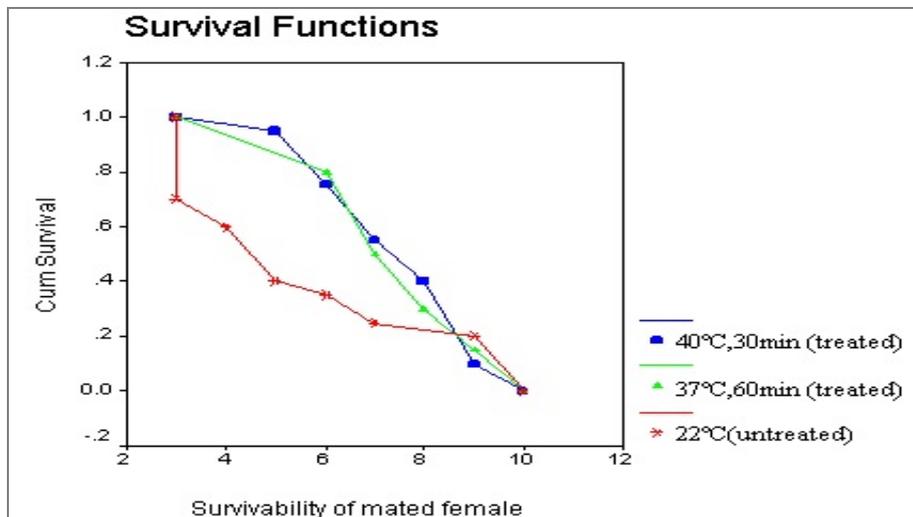


Fig 6: Survival curve of mated females for untreated and treated groups

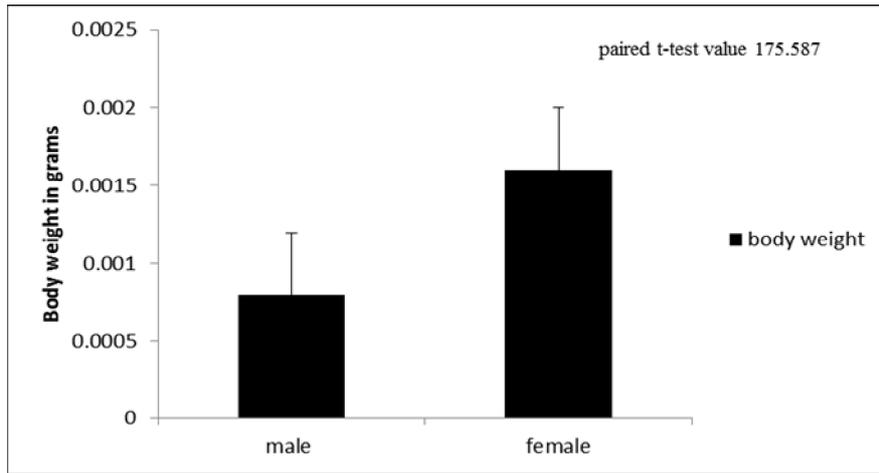


Fig 7: Body weights of male and female *D. melanogaster*

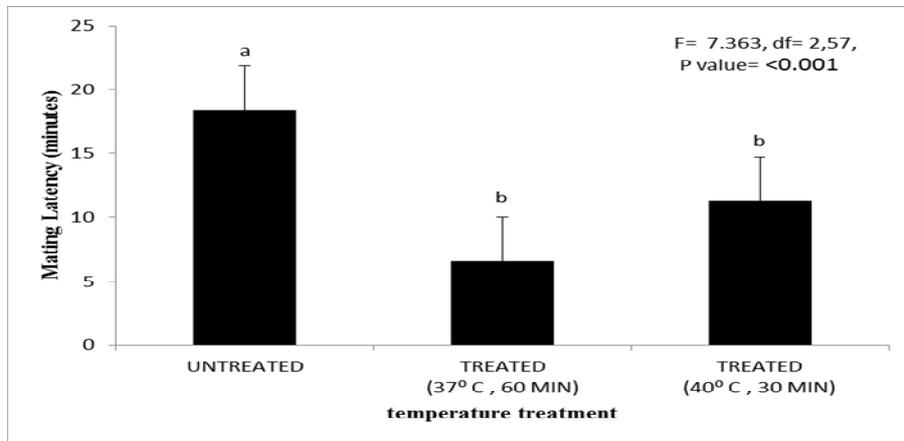


Fig 8: Mating latency of untreated and treated males of *D. melanogaster*. Different letters on bar graph indicate significance at $p < 0.05$ level by Tukey's post hoc test

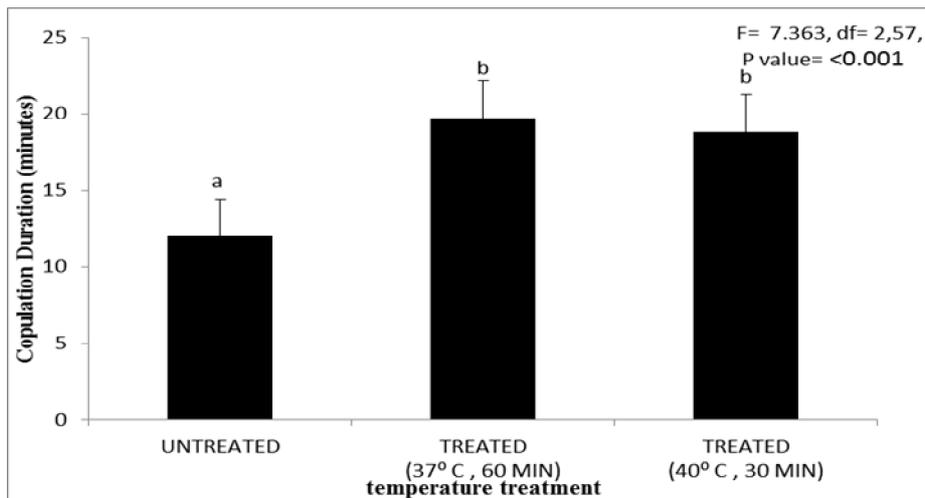


Fig 9: Copulation duration of untreated and treated males of *D. melanogaster*. Different letters on bar graph indicate significance at $p < 0.05$ level by Tukey's post hoc test

Table 1: Mantel-Cox, Generalised Wilcoxon and Tarone-Ware Chi-Square test for Unmated and mated flies of *D. melanogaster*.

		Chi-Square	Df	Sig.
Unmated males	Log Rank (Mantel-Cox)	2.637	2	.267
	Breslow (Generalized Wilcoxon)	8.141	2	.017
	Tarone-Ware	5.330	2	.070
Mated males	Log Rank (Mantel-Cox)	19.179	2	.000
	Breslow (Generalized Wilcoxon)	27.939	2	.000
	Tarone-Ware	23.788	2	.000
Unmated females	Log Rank (Mantel-Cox)	6.602	2	.037
	Breslow (Generalized Wilcoxon)	21.573	2	.000
	Tarone-Ware	13.989	2	.001
Mated females	Log Rank (Mantel-Cox)	3.728	2	.155
	Breslow (Generalized Wilcoxon)	4.991	2	.082
	Tarone-Ware	4.443	2	.108

Table 2: Quantity of Acps in untreated and treated, mated males of *D. melanogaster*

Sources	quantity of Acps		
	Unmated	Mated	Quantity of transfer
Untreated	20.32±.39 ^a	14.38±.42 ^a	5.93±.16 ^a (29.23%)
Short term sub lethal temperature treated at 37 °C for 60 min	18.87±.17 ^b	10.79±.17 ^b	8.07±.26 ^b (42.76%)
Short term sub lethal temperature treated at 40 °C for 30 min	18.16±.10 ^b	9.84±0.04 ^b	8.31±.10 ^b (45.75%)
F-value	18.303*	81.590*	47.634*

* p<0.001 level, Different letters on mean values indicate significance at p<0.05 level by Tukey's post hoc test.

4. Results

4.1 Effect of short term temperature on Starvation resistance of unmated and mated flies

Data for starvation resistance among untreated and treated flies is shown in figures 1-6. Starvation resistance among mated males was found to be greater than the unmated males in treated groups while in untreated group, unmated males had greater starvation resistance than mated males (Figure 1, 3 and 4). Among unmated males starvation resistance was found to be more or less similar in both the groups. While in mated males starvation resistance was higher in treated group compared to untreated group. One way ANOVA followed by Tukey's post hoc test showed insignificant variation between unmated males but showed significant variation between mated males (Figure 1). Tukey's post hoc test showed that resistance among flies treated with 40 °C for 60 mins was significantly higher compared to untreated. Among the treated groups, a slight insignificantly greater starvation resistance was found in 37 °C treated group than 40 °C treated as showed by Tukey's post hoc test. Similar results were also obtained in survival curve (Table 1)

Starvation resistance of mated females was found to be higher than unmated females in treated groups, while in untreated group, unmated females had lower starvation resistance than mated females (Figure 2, 5 and 6). Among unmated females, starvation resistance was found to be more or less similar in untreated and treated groups. While in mated females starvation resistance was higher in treated groups compared to untreated groups. One way ANOVA followed by Tukey's post hoc test showed insignificant variation between unmated females but showed significant variation between mated females. Tukey's post hoc test showed that resistance among flies treated with 37 °C for 60 mins and flies treated with 40 °C for 30 mins was insignificantly higher and

similar.

4.2 Body weight of male and female flies.

Figure 7 shows that females of *D. melanogaster* had greater body weight compared to the body weight of males. Paired t-test carried out on the above data showed significant variation between males and females.

4.3 Effect of short term sub lethal temperature on Mating Latency (ML) and Copulation Duration (CD)

Mean values of mating latency (ML) and copulation duration (CD) of untreated and treated males of *D. melanogaster* are shown in figure 8 and 9. Time taken for mating was found to be highest in untreated compared to treated groups. Among treated group, mating latency increased with increase in temperature treatments. One way ANOVA followed by Tukey's post hoc test carried out on mating latency data showed significant variation (Figure 8). Untreated flies had significantly greater mating latency compared to treated flies as given by Tukey's test. Time taken for mating latency was insignificantly greater in temperature treatment of 40 °C compared to flies of 37 °C treatments by Tukey's test.

Copulation duration was found to be lowest in untreated compared to treated groups. Among treated groups, copulation decreased with increase in short term temperature treatments. One way ANOVA followed by Tukey's post hoc test carried out on copulation duration data showed significant variation (Figure 9). Untreated flies had significantly lower copulation duration compared to treated flies as shown by Tukey's test. Copulation duration was insignificantly greater in short term temperature treatment of 37 °C compared to flies of 40 °C treatments by Tukey's test.

4.4 Effect of short term temperature on quantity of Acps in unmated and mated flies

Quantity of Acps in both mated and unmated males (Table 2) decreased with increase in temperature. Highest quantity of Acps was found in untreated flies. Among the treated flies quantity of Acps was found to be greater in 37 °C treated flies than flies treated at 40 °C. One way ANOVA followed by Tukey's post hoc test showed significant variation in quantity of Acps in untreated and treated flies. Tukey's test showed that quantity of Acps was significantly greater in untreated compared to flies treated with 37 °C 60 mins and 40 °C for 30 min. Further, quantity of Acps was found to be insignificantly greater in flies treated with 37 °C for 60 mins than flies treated with 40 °C for 30 mins as observed by Tukey's post hoc test.

The transferred quantity of Acps was determined by calculating the difference of Acps quantity between unmated and mated flies. The transfer was high in treated flies compared to untreated flies (Table 2). Transfer rate was 42.76% in flies treated with 37 °C for 60mins and 45.75% in flies treated with 40 °C for 30mins. In untreated group the transfer was only 29.23%.

5. Discussion

The greater resistance to starvation among females of *D. melanogaster* than males as observed in our study suggests the existence of sexual dimorphism with reference to starvation resistance. Our result confirms the works of Goenaga ^[13] while works on *D. melanogaster* have also demonstrated that mated females exhibit an increased tolerance to starvation in comparison to virgins. This resistance in females could be attributed to the increased lipid reserves which may account for the greater body weight of females than males ^[2, 15, 27]. Even in our study the body weight of female was significantly greater compared to body weight of the male. Alternative mechanisms to increase resistance to starvation may be the reduction in utilization of reservoirs or lowering of minimal body energy content which allows the survival ^[27]. Also, the short term temperature treatments showed significant influence on resistance to starvation.

In nature, temperature rises more slowly than in most laboratory experiments, and extremities usually will not differ greatly from day to day. Therefore, most organisms encountering high temperatures previously will have activated the heat shock response and the genetic variation in resistance to short-term exposure to a high temperature that is relevant for adapting to a warmer environment. With exposure to high temperatures, survival will depend on the ability to withstand the stress physiologically, with selection acting on genetic variation either for the quantity of some heat shock protein produced, for its amino-acid sequence, or for thermal stability of structural or enzymatic proteins. With increase in either the mean temperature or its variance, populations will more likely become exposed to short-term temperature stress and the amount of genetic variation present within a population for stress resistance may be an indicator of how that population will adapt. Unlike *D. buzatii*, a cactophilic species which may commonly be exposed to high temperatures, *D. melanogaster*, a domestic species does not encounter such a high temperature in domestic localities. As a result it will not show any genetic variation to heat resistance when compared to *D. buzatii*. Further, *Kreb et al.* ^[10] have shown that a threshold for thermal stress exists in *D. melanogaster* above which the fitness of individuals may be reduced greatly, and local extinction of population may be more likely than evolutionary adaptation for resistance. Therefore more studies are required in

other species of *Drosophila* to rule out the possible benefits obtained from short term sub lethal heat stress.

As thought earlier, mating may not be a harmful process but could be beneficial to females with reference to resistance to starvation. Therefore it has been hypothesized that increased mating rate is advantageous for the females in *D. melanogaster*. The increased resistance to starvation in mated males and females may be due to the accessory gland proteins transferred by males to females along with seminal gland proteins during copulation. Accessory glands produce and secrete a complex mixture of proteins that form components of the seminal fluid, which is transferred along with sperm to the female during copulation ^[15] ^[25, 35]. In mated females, accessory gland proteins (Acps) induce physiological and behavioral changes. They enhance the rate of egg production, ovulation, reduces her sexual receptivity, assist in the female's storage of sperm and also contribute to the reduced life span of the mated female ^[35, 12]. The quantity of Acps in unmated males was highest in untreated males than the treated males; among the treated males quantity of Acps was found to be higher in 37 °C than in flies treated with 40 °C suggesting that as temperature increases quantity of Acps decreases. Acps of mated males also showed similar results. As observed, the females mating with treated males obtains greater quantities of Acps as which confers them the greater resistance to starvation.

This study also shows that the mating latency is higher in untreated flies than treated flies and the duration of copulation is longer in treated flies than the untreated flies. The treated flies with their fast mating capabilities, had copulated for a significantly longer time and transferred greater quantity of Acps as a result of which, the females mated with treated males had greater resistance to starvation compared to untreated flies.

Studies in insect groups have shown that females mate with multiple males during periods of starvation and desiccation. This multiple mating helps females to acquire larger amounts of water and nutrients that may improve female starvation resistance ^[3, 10, 17]. In *D. mojavensis* it was shown that males transfer nuptial gifts that could help the females to overcome periods of food deprivation ^[26]. Studies in *D. melanogaster* by *Goenaga et al.* ^[13] showed the pivotal role of mating and they supported the hypothesis that Acps transfer during mating helps the females to enhance their resistance to starvation. As far as our knowledge goes, no studies are made to quantify Acps and our study stands as an evidence to support this hypothesis. These results well establish the fact that during extreme thermal and food deprivation conditions, mating is highly beneficial for females which helps by conferring resistance to starvation. This study rules out the idea that mating may impair female fitness as proposed earlier.

6. Acknowledgement

Authors are grateful to the Chairman, DOS in Zoology and M.Sc Genetics Course Coordinator for providing the facilities to carry out this project work. Authors are also grateful to *Drosophila* Stock center for providing *Drosophila* stocks and other facilities.

7. References

1. Alexandra GI, Volker L, Lex A, Zhivotovsky, Oleg EL. Stress temperatures and quantitative variation in *Drosophila melanogaster*. *Heredity* 1998; 81:246-253.
2. Ballard JWO, Melvin RG and Simpson SJ. Starvation resistance is positively correlated with body lipid proportion in five wild caught *Drosophila simulans* populations. *Journal of Insect Physiology* 2008;

- 54:1371-1376.
3. Boucher L, Huignard J. Transfer of male secretions from the spermatophore to the female insect *Caryedon serratus*: analysis of the possible trophic role of these secretions. *Journal of Insect Physiol* 1987; 33:949–957
 4. Bublly OA, Loeschcke V. High stressful temperature and genetic variation of five quantitative traits in *Drosophila melanogaster*. *Genetica* 2001; 110:79-85.
 5. Chen PS. The functional morphology and biochemistry of insect male accessory glands and their secretions. *Annu Rev Entomol* 1984; 29:233–55.
 6. Dahlggaard J, Loeschcke V, Michalak P, Justesen J. Induced thermotolerance and associated expression of the heat-shock protein Hsp70 in adult *Drosophila melanogaster*. *Funct Ecol* 1998; 12:786–793.
 7. David RJ, Gibert P, Moreteau B, Gilchrist GW, Huey RB. The fly that came in from the cold: geographic variation of recovery time from low-temperature exposure in *Drosophila subobscura*. *Functional Ecology* 2003; 17:425–430.
 8. David JR, Allemand R, Van-Herrewege J, Cohet Y. Ecophysiology: Abiotic factors. In: Ashburner, M, Carson, HL and Thompson JN (eds) *The genetics and biology of Drosophila* Academic press, London. 1983; 3:105-170
 9. David J, Clavel MF. Influence de la temperature sur le nombre, le pourcentage d'eclosion et la taille des oeufs fondus par *D. melanogaster*. *Ann Soc Entomol* 1969; 5:161-177.
 10. Edvardsson M. Female *Callosobruchus maculatus* mate when they are thirsty: resource-rich ejaculates as mating effort in a beetle. *Animal Behavior* 2006; 74:183–188.
 11. Ghalambor CK, McKay JK, Carroll SP, Reznick DN. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct Ecol* 2007; 21:394-407.
 12. Gillott C. Male accessory gland secretions: Modulators of Female Reproductive Physiology and Behavior. *Annu Rev Entomol* 2003; 48:163-184.
 13. Goenaga J, Mensch J, Fanara JJ, Hasson E. The effect of mating on starvation resistance in natural populations of *Drosophila melanogaster*. *Evol Ecol* 2011. DOI 10.1007/s10682-011-3540-4.
 14. Hercus MJ, Loeschcke V, Rattan SIS. Lifespan extension of *Drosophila melanogaster* through hormesis by repeated mild heat stress. *Biogerontology* 2003; 4:149–156.
 15. Hoffmann AA, Hallas R, Anderson AR, Telonis-Scott M. Evidence for a robust sex-specific trade-off between cold resistance and starvation resistance in *Drosophila melanogaster*. *J Evol Biol* 2005; 18:804–810.
 16. Hoffmann AA, Sorensen JG, Loeschcke V. Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *J Thermal Biol* 2003; 28:175-216.
 17. Hoffmann AA, Harshman LG. Desiccation and starvation resistance in *Drosophila*: patterns of variation at the species, population, and intrapopulation levels. *Heredity* 1999; 83:637–643.
 18. Hoffmann AA, Parsons PA. *Evolutionary Genetics and Environmental Stress*. Oxford University Press 1991, Oxford.
 19. Huey RB, Wakefield T, Crill WD, Gilchrist GW. Within and between generation effects of temperature on early fecundity of *Drosophila melanogaster*. *Heredity* 1995; 74:216-223.
 20. Kingsolver JG. Feeding, growth, and the thermal environment of cabbage white caterpillars, *Pieris rapae l.* *Physiol Biochem Zool* 2000; 73:621-628.
 21. Krebs RA, Loeschcke V. Effects of exposure to short-term heat stress on fitness components in *Drosophila melanogaster*. *Journal of Evolutionary Biology* 1994; 7:39-49.
 22. Levins R. Thermal acclimation and heat resistance in *Drosophila* species. *American Naturalist* 1969; 103:483-499.
 23. Markov TA, O'Gardy PM. Evolutionary genetics of reproductive behavior in *Drosophila*; connecting the dots. *Annu Rev Genet* 2005; 39:263-291.
 24. Mckenzie JA. The influence of low temperature on survival and reproduction in populations of *Drosophila melanogaster*. *Aust J Zool* 1975; 23:237-247.
 25. Monsma SA, Wolfner MF. Structure and expression of a *Drosophila* male accessory gland gene whose product resembles a peptide pheromone precursor. *Genes Dev* 1988; 2:1063–73.
 26. Pitnick S, Spicer GS, Markow TA. Phylogenetic examination of female incorporation of ejaculate in *Drosophila*. *Evolution* 1997; 5:833–845
 27. Rion S, Kawecki TJ. Evolutionary biology of Starvation resistance; What we have learned from *Drosophila*. *J evol Biol* 2007; 20:1655-1664.
 28. Rose MR, Vu LN, Park SU, Graves JL. Selection on stress resistance increases longevity in *Drosophila melanogaster*. *Exp Gerontol* 1992; 27:241±250.
 29. Schmidt TES, Kachman SD, Harshman LG. Evolution of starvation resistance in *Drosophila melanogaster*: measurement of direct and correlated responses to artificial selection. *Journal of Evolutionary Biology* 2012; 25:378-387.
 30. Schnebel SM, Grossfield J. Oviposition temperature range in 4 *Drosophila* species triads from different ecological backgrounds. *Am Midl Nat* 1986; 116:25-35.
 31. Service PM, Hutchinson EW, Mackinley MD, Rose MR. Resistance to environmental stress in *Drosophila melanogaster* selected for postponed senescence. *Physiol. Zool* 1985; 58:380–389.
 32. Siddiqui W, Barlow C. Population growth of *Drosophila melanogaster* (Diptera: Drosophilidae) at constant and alternating temperatures. *Ann Entomol Soc Am* 1972; 65:993-1001.
 33. Sisodia S, Singh BN. Effect of exposure to short-term heat stress on survival and fecundity in *Drosophila ananassae*. *Can J Zool* 2006; 84:895-899.
 34. Sørensen JG, Kristensen TN, Loeschcke V. The evolutionary and ecological role of heat shock proteins. *Ecology Letters* 2003; 6:1025–1037
 35. Wolfner MF. The gifts that keep on giving: physiological functions and evolutionary dynamics of male seminal proteins in *Drosophila*. *Heredity* 2002; 88:85–93.
 36. Zani PA, Swanson SET, Corbin D, Cohnstaedt LW, Agotsch MD, Bradshaw WE *et al.* Geographic variation in tolerance of transient thermal stress in the mosquito *Wyeomyia smithii*. *Ecology* 2005; 86:1206-1211.