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Tissue tolerance to heat and cold shock in larvae of *Sarcophaga ruficornis* (Sarcophagidae: Diptera)

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ABSTRACT
Larvae of the flesh fly, *Sarcophaga ruficornis* were exposed to Heat and Cold shock stresses in the present study. One of the responses to stress is the internal tissue damage. Trypan blue dye exclusion method was used to analyze the tissue tolerance to heat shock and cold shock. There is a difference in tolerance level in nongut and gut tissues to these thermal stresses.

Keywords: Flesh fly, Trypan blue, Temperature stress, Tissue damage.

1. Introduction

Temperature is one of the most important environmental factors influencing physiological function of organisms. The physiological response to stress correlates with expression of genes whose proteinaceous product presumably contribute to protection from injury due to stress[1-3].

Studies on tissue damage by various stress responses have been limited to only a few dipterans, viz. *Drosophila melanogaster*[4, 5], transgenic *D. melanogaster*[6-13], *Eurosta solidaginis*[14], *Chilo* suppressalis[15], *Delia antique*[16, 17], *Belgica antarctica*[18], *Lucilia cuprina*[19] and *Musca domestica*[20].

Trypan blue dye exclusion assay is the most commonly used method for measurement of cell viability[15]. The method is based on the principle that live or viable cells do not take up trypan blue while it readily enters dead or non-viable cells. In the present study, trypan blue dye exclusion method has been used to analyze the tissue damage due to heat shock and cold shock in larvae of *Sarcophaga ruficornis* (Sarcophagidae: Diptera). Capacity of larvae to survive after heat and cold shock has also been assessed by analyzing mortality of larvae at high and low temperature.

2. Material and Methods

Laboratory stocks of *Sarcophaga ruficornis* Fab. (Sarcophagidae: Diptera) was used to analyze the tissue damage in larvae in response to heat and cold shock. The flies were reared in the laboratory following the method of Kaul and Tewari[21]. Response of larval tissues of *S. ruficornis* to heat shock and cold shock was examined by staining with Trypan Blue.

2.1. Heat shock

Five sets of 10 late third instar larvae were placed in a test tube lined with moist tissue paper, covered with muslin cloth and exposed to 40 ºC for 30 min. and 60 min. For control 10 late third instar larvae were placed in a test tube covered with muslin cloth and kept at room temperature (26±2 ºC) for 30 min. and 60 min.

2.2. Cold shock

Five sets of 10 late third instar larvae were placed in a test tube covered with muslin cloth and placed in Deep freezer at -10 ºC and -20 ºC for 30 min. and 60 min. For control 10 late third instar larvae were placed in a test tube covered with muslin cloth and kept at room temperature (26±2 ºC) for 30 min. and 60 min.

2.3. Analysis of damaged tissue

The control and treated larvae were dissected and the internal tissues were immersed for 30 min. at room temperature in Trypan Blue (0.2 mg/ml) in Phosphate Buffer Saline (PBS) (pH 7.4). The tissues were intermittently rotated.

Tissues were rinsed thrice in PBS, washed for 30 min. in PBS and immediately scored for Trypan Blue staining following the method of Krebs and Feder[4].

2.4. Mortality of larvae at high and low temperature

Mortality rate was observed after heat shock (40 °C) and cold shock (-20 °C) for different time intervals i.e.15 min, 30 min., 45 min & 60 min. Following exposure larvae were thawed at room temperature and the mortality rates were assessed after 1 hour. Moving larvae were designated as survivors and the rest were considered as dead.

3. Observation

The staining patterns of different larval tissues after heat and cold shock are summarized in Table 1.

Table 1: Staining pattern of control and stressed larval tissues of *S. ruficornis*.

	Bg	Sg	Gc	Mg	Hg	Mt
Control	+	-	+	-	+	+
Heat Shock (40 °C/30 min.)	++	+	++	+++	++	++++
Heat Shock (40 °C/60 min.)	++	++	++	+++	+++	++++
Cold Shock (-10 °C /30 min.)	++	++	+++	++++	+++	++++
Cold Shock (-10 °C /60 min.)	++	++	+++	++++	+++	++++
Cold Shock (-20 °C /30 min.)	++	++	+++	++++	+++	++++
Cold Shock (-20 °C /60 min.)	++	++	+++	++++	+++	++++

Bg-Brain ganglia, Sg - Salivary gland, Gc - Gastric caeca, Mg - Mid gut, Hg –Hind gut , Mt - Malpighian tubule:
(-)= No staining, (+) = Pale blue staining, (++) = Moderate staining, (+++) Darker staining, (++++) = Darkest staining.

3.1. Tissue damaged in larvae after heat shock

Heat shock treatment, at 40 °C for 30 min. and 60 min. to the larvae show darker staining in brain ganglia, salivary gland, gastric caeca, mid gut, hind gut and malpighian tubule as compared to control. The brain ganglia, salivary gland and gastric caeca show moderate staining, while hind gut reveals dark staining. Malpighian tubule and midgut show the darkest staining (Fig.1a and b).

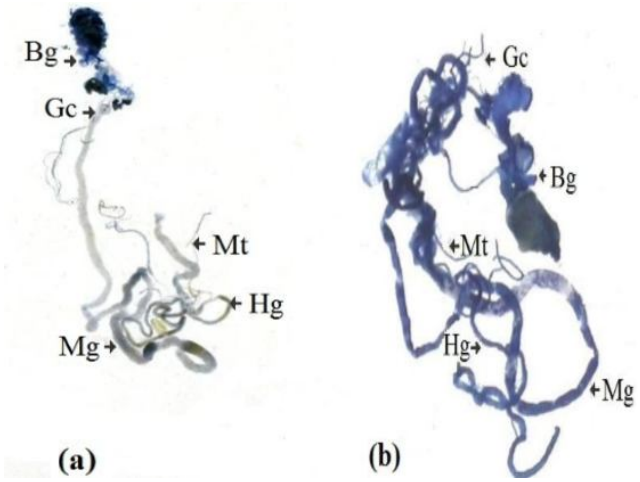

Fig 1: Trypan blue staining pattern in third instar larval tissues of *S. ruficornis* (a) Control (b) *in vivo* heat shock at 40 °C /60 min.

3.2. Tissue damaged in larvae after cold shock

Cold shock at -10 °C for 30 min. and 60 min., elicits a response which is similar to heat shock, in brain ganglia, salivary gland and malpighian tubule. However, the gastric caeca and hind gut show darker staining and midgut shows darkest staining as compared to heat shock response.

Similar findings were observed with that the cold shock at -20 °C for 30 min. and 60 min. The intensity of staining was strongest in midgut and malpighian tubules which mean that these tissues show lowest tolerance to temperature stress (Fig 2 a and b).

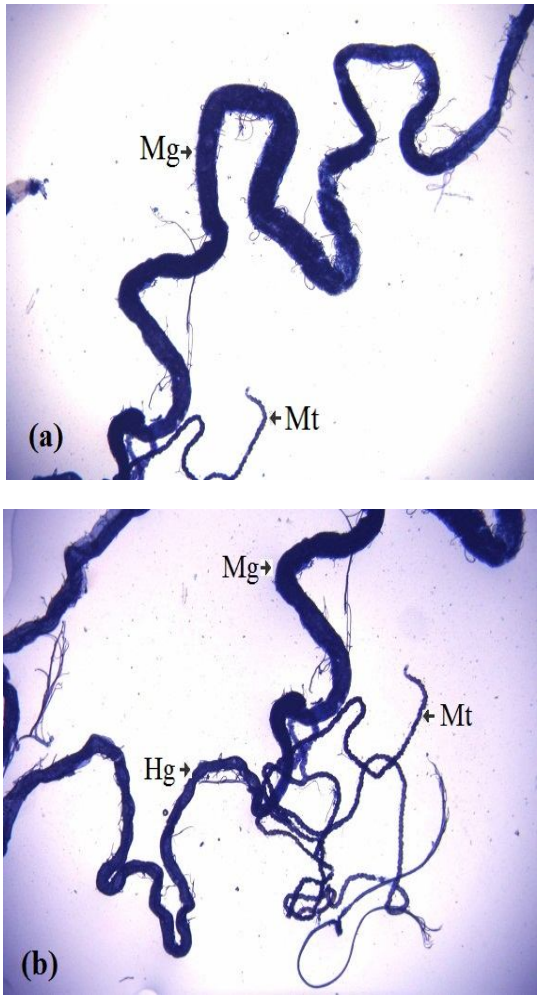

Fig 2: (a) Cold shock effect in mid gut (b) Cold shock effect in hind gut and malpighian tubule in third instar larvae of *S. ruficornis*.

3.3. Mortality rate after heat and cold shock

The rate of mortality increased with treatment time, though the rate of mortality was low in heat shock. After 60 min. exposure to heat shock temperature and cold shock temperature the mortality is approximately 100%. The mortality rate of *S. ruficornis* larvae after heat and cold shock is represented in Fig.3.

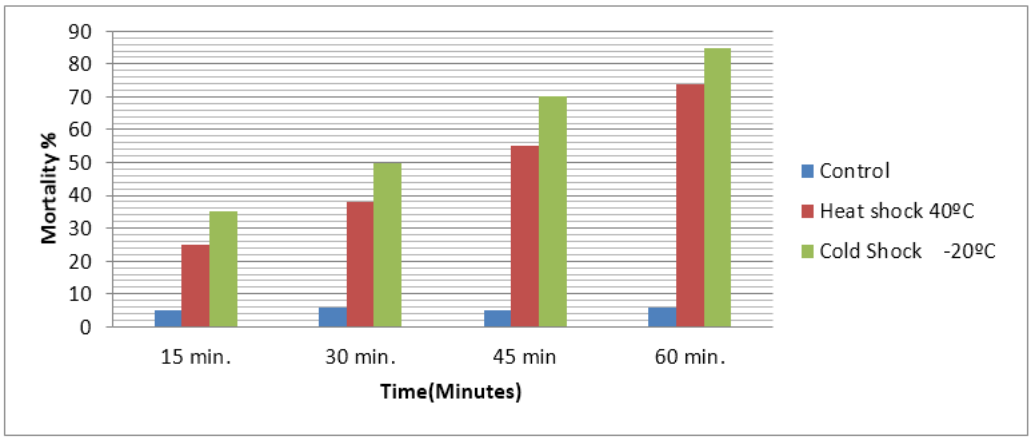

Fig 3: Effect of heat and cold shock on mortality of *S. ruficornis* larvae.

4. Discussion

Animal tissues are prone to Heat and Cold shock[4, 22]. The integumentary muscles, haemocytes, trachea and some portion of the malpighian tubules have been found to be more susceptible to cold shock injury in overwintering larvae of *Eurosta* solidiganis[14]. However, in non diapausing larvae the midgut, silk gland and fat bodies are susceptible to freezing injury[15]. Krebs and Feder[4] opined that gut tissues show extensive necrosis after heat stress as revealed by trypan blue staining which increased in all gut tissues viz., salivary gland, gastric caeca, mid gut, hind gut as compared to nongut tissues. In *Sarcophaga ruficornis* larvae where the midgut, hindgut and malpighian tubules are more susceptible to heat and cold shock as compared to brain ganglia and salivary gland. Malpighian tubules and the gut constitute the primary system for ion regulation, osmoregulation and excretion in insects. These organs may be especially valuable model for studying the effect of freezing injury and heat shock[23, 24].

A variety of thermal protective mechanisms may act in synchrony to prevent cellular damage[25].The general stress response involves the expression of stress proteins (Hsp). Their induction is often accompanied by tolerance to these stresses[26-31]. It seems that hsp70 in higher eukaryotes plays a central role in thermotolerance [32, 33].

Lindquist[34, 35] found in *D. melanogaster* that hsp 70 is the primary inducible heat shock protein and is not expressed before stress and even Hsp70 expression is strongly repressed in the absence of heat shock or once recovery from heat shock is complete. There is a close link between Hsp 70 expression and tissue damage as revealed by trypan blue staining[36]. According to Krebs and Feder[4] Hsp 70 expression and trypan blue staining may represent thermo sensitive region within an organism. They observed that the tissue which showed a prolonged delay in Hsp 70 expression after shock i.e. gut, stained intensely with trypan blue suggesting that the gut tissue is especially thermosensitive. The increases and decreases in Hsp 70 concentration is known to affect thermotolerance[33, 37, 38]. In *D. melanogaster* tissues such as brain and salivary glands which rapidly produced Hsp 70 in response to high temperatures suffered less damage from heat shock as compared to tissues namely caeca and midgut which show slow expression of Hsp 70 revealed extensive thermal damage[4, 39] .

In *S. ruficornis* larvae the brain ganglia, salivary gland suffered less damage during temperature stress in comparison to mid gut , hind gut and malphigian tubules as revealed by trypan blue staining. Thus it seems that in *S. ruficornis* larvae nongut tissue i.e. salivary gland and brain ganglia have high tolerance and gut and malpighian tubules have low tolerance to temperature stress.

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6. Reference

1. Deshaies RJ, Koch BD, Werner-Washburne M, Craig EA, Schekeman R. A subfamily of stress proteins facilitates translocation of secretory and mitochondrial precursor polypeptides. Nature 1988; 332:800-805.
2. Chirico WJ, Waters MG, Blobel G. 70 k heat shock related proteins stimulate protein translocation into microsomes. Nature 1988; 332:805-810.
3. Chiang HL, Terlecky SR, Plant CP, Dice JF. A role for a 70-kilodalton heat shock protein in lysosomal degradation of intracellular proteins. Science 1989; 246:382-385.
4. Krebs RA, Feder ME. Tissue specific variation in Hsp 70 expression and thermal damage in *Drosophila melanogaster* larvae. Journal of Experimental Biology 1997; 200:2007-2015.
5. Mukhopadhyay I, Siddique HR, Bajpai VK, Saxena DK, Chowdhuri DK. Synthetic pyrethroid cypermethrin induced cellular damage in reproductive tissues of *Drosophila melanogaster*: Hsp70 as a marker of cellular damage. Arch Environ Contam Toxicol 2006; 51(4):673-80.
6. Chowdhuri DK, Nazir A, Saxena DK. Effect of three chlorinated pesticides on hsromega stress gene in transgenic *Drosophila melanogaster*. J Biochem Mol Toxicol 2001; 15(4):173-86.
7. Mukhopadhyay I, Nazir A, Mahmood K, Saxena DK, Das M, Khanna SK, Chowdhuri DK. Toxicity of argemone oil: effect on hsp70 expression and tissue damage in transgenic *Drosophila melanogaster* (hsp70-lacZ) Bg9. Cell Biol Toxicol 2002; 18(1):1-11.
8. Mukhopadhyay I, Saxena DK, Bajpai VK, Chowdhuri DK. Argemone oil induced cellular damage in the reproductive

tissues of transgenic *Drosophila melanogaster*: protective role of 70 kDa heat shock protein. J Biochem Mol Toxicol 2003; 17(4):223-34.
9. Nazir A, Mukhopadhyay I, Saxena DK, Siddiqui MS, Chowdhuri DK. Evaluation of toxic potential of captan: Induction of hsp70 and tissue damage in transgenic *Drosophila melanogaster* (hsp70-lacZ) Bg9. J Biochem Mol Toxicol 2003; 17(2):98-107.
10. Gupta SC, Siddique HR, Saxena DK, Chowdhuri DK. Hazardous effect of organophosphate compound, dichlorvos in transgenic *Drosophila melanogaster* (hsp70-lacZ): induction of hsp70, anti-oxidant enzymes and inhibition of acetylcholinesterase. Biochim Biophys Acta. 2005; 1725(1):81-92.
11. Gupta SC, Siddique HR, Mathur N, Mishra RK, Chowdhuri DK. Adverse effect of organophosphate compounds, dichlorvos and chloropyrifos in the reproductive tissues of transgenic *Drosophila melanogaster*:70 KDa heat shock protein as a marker of cellular damage.Toxicology 2007; 238:1-14.
12. Kumar V, Ara G, Afzal M, Siddique YH. Effect of methyl methanesulfonate on hsp70 expression and tissue damage in the third instar larvae of transgenic *Drosophila melanogaster* (hsp70-lacZ) Bg. Interdiscip Toxicol 2011; 4(3):159-65.
13. Shakya B, Jyoti S, Naz F, Khan S, Afzal RM, Siddique YH. Effect of L-ascorbic Acid on the hsp70 Expression and Tissue Damage in the Third Instar Larvae of Transgenic *Drosophila melanogaster* (hsp70-lacZ) Bg(9). Toxicol Int 2012; 19(3):301-5.
14. Yi SX, Lee RE. Detecting freeze injury and seasonal cold-hardening of cells and tissues in the gall fly larvae, *Eurosta solidaginis* (Diptera: Tephitidae) using fluorescent vital dyes. J Insect Physiol 2003; 49:999-1004.
15. Izumi Y, Sonoda S, Yoshida H, Tsumuki H. Identification of tissues showing the lowest tolerance to freezing in larvae of rice stem borer, *Chilo suppressalis*. Physiological Entomology 2005; 30:324-331.
16. Kyukawa T, IshikawaY. Detection of chill injuries in the pupae of the onion maggot, *Delia antiqua* (Diptera: Anthomyiidae). Appl Entomol Zool 2005; 40(1):193-198.
17. Kyukawa T, Ishikawa Y. Chaperonin contributes to cold hardiness of the onion maggot *Delia antiqua* through repression of depolymerization of actin at low temperatures. PLos ONE 2009; 4(12):e8277(1-7).
18. Nicholas MT, Michael AE, Joshua BB, Giancarlo LM, Denlinger DL, Lee RE Jr. Rapid cold hardening in larvae of the Antarctic midge *Belgica antarctica*: Cellular cold-sensing and a role for calcium. Am J Physiol Regul Integr Comp Physiol 2008; 294:R1938-1946.
19. Sharma S, Rohilla MS, Reddy PV and Tiwari PK. In vitro induction of 60-kDa and 70-kDa heat shock proteins by endosulphan and monocrotophos in sheep blowfly *Lucilia cuprina*.Arch Environ Contam Toxicol. 2008; 55(1):57-69.
20. Mishra N, Tewari RR. Cytotoxic and genotoxic effects of mercury in house fly *Musca domestica* (Diptera: Muscidae). Cell Mol Biol 2011; 57(1):122-128.
21. Kaul D, Tewari RR. Interspecific comparisons of polytene chromosomes in the genus *Parasarcophaga* (Sarcophagidae: Diptera). Genetica 1983; 62:129-138.
22. Kasai M. Ito K, Edashige K. Morphological appearance of the cryopreserved mouse blastocyst as a tool to identify the type of cryoinjury. Human Reproduction, 2002; 17:1863-1874.
23. O'Donnell MJ, Spring JH. Modes of control of insect malpighian tubules: synergism, antagonism, cooperation and autonomous regulation. J Insect Physiol 2000; 46(2):107-117.
24. Zeiske W. Insect ion homeostasis. J Exp Biol 1992; 172:323-334.
25. Feder ME, Hoffmann GE. Heat- shock proteins, molecular chaperones and the stress response: Evolutionary and ecological physiology. Annu Rev Physiol 1999; 61:243-283.
26. Burton V, Mitchell HK, Young P, Petersen NS. Heat shock protection against cold stress of *Drosophila melanogaster*. Mol Cell Biol 1988; 8:3550-3552.
27. Joplin KH, Yocum GD, Denlinger DL. Cold shock elicits expression of heat shock proteins in the flesh fly *Sarcophaga crassipalpis*. J Insect Physiol 1990; 36:825-834.
28. Yiangou M, Tsapogas P, Nilkolaidis N, Scouras ZG. Heat shock gene expression during recovery after transient cold shock in *Drosophila auraria* (Diptera: Drosophilidae). Cytobios 1997; 92:91-98.
29. Goto SG, Kimura MT. Heat and cold-shock responses and temperature adaptations in subtropical and temperate species of *Drosophila*. J Insect Physiol 1998; 44:1233-1239.
30. Sejerkilde M, Sorensen JG, Loeschcke V. Effects of heat and cold hardening on thermal resistance in *Drosophila melanogaster*. J Insect Physiol 2003; 49:719-726.
31. Nielsen MM, Overgaard J, Sorensen JG, Holmstrup M, Justesen J, Loeschcke V. Role of heat shock factor during heat and cold hardening and for the resistance to severe heat and cold stress. J Insect Physiol 2005; 51:1320-1329.
32. Hahn G, Li G. In *Stress Proteins in Biology and Medicine*. Edited by Morimoto R, Tissieres A, Georgopoulos C. Cold Spring Harbor, Cole Spring Harbor Laboratory Press: New York, 1990, 79-100.
33. Solomon J, Rossi JM, Golic K, McGarry T, Lindquist S. Changes in Hsp 70 alter thermotolerance and heat-shock regulation in *Drosophila*. New Biol 1991; 3:1106-1120.
34. Lindquist S. Regulation of protein synthesis during heat shock. Nature 1981; 293:311-314.
35. Lindquist S. Autoregulation of the heat-shock response. In. *Transational Regulation of Gene Expression* 2 (ed. J IIan). Plenum Press, New York 1993, 279-320.
36. Ryan JA, Hightower LE. Stress proteins as molecular biomarker for environmental toxicology. Stress-inducible Cellular Responses (ed. Fiege RI, Yahara I and Polla B). Basel, Switzerland, Birkhauser, 1996, 411-424.
37. Welte MA, Tetrault JM, Dellavalle RP, Lindquist SL. A new method for manipulating transgenes: Engineering heat tolerance in a complex, multicellular organism. Curr Biol 1993; 3:842-853.
38. Feder ME, Cartano NV, Milos L, Krebs RA, Lindquist SL. Effect of engineering hsp70 copy number on Hsp 70 expression and tolerance of ecologically relevant heat shock in larvae and pupae of *Drosophila melanogaster*. J Exp Biol 1996; 199:1837-1844.
39. Feder ME, Krebs RA. Natural and Genetic Engineering of the Heat-Shock Protein Hsp70 in *Drosophila melanogaster*: Consequences for thermotolerance. Amer Zool 1998; 38:503-517.