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Chemical stress response of polytene chromosomes of *Parasarcophaga ruficornis* (Sarcophagidae : Diptera)

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ABSTRACT

Stress response to sodium azide, 2-4 dinitrophenol and benzamide was analyzed in polytene chromosomes of pupal foot pad cell of *Parasarcophaga ruficornis.* Stress was given for different time intervals. In each case, only a single large puff was induced in chromosome arm II L at the region 12A. The same puff was induced in other Sarcophagid species by heat and chemical stress. It appears that a single conserved prominent puff is a hall mark of the stress response in Sarcophagids.

Figures : 03 References : 31 Tables : 03
KEY WORDS : Banda, Chemical stress, *P. ruficornis*

Introduction

The polytene chromosomes have served as an outstanding model for a variety of genetic experiments. Using the polytene chromosomes, the stress response of the genome at a cytological level has been studied. Ritossa in mid 1960's initially analyzed the cellular response to environmental stress in salivary gland chromosomes of *Drosophilla busckii*, which unraveled a novel set of chromosomes puffs. An extensive study of heat shock response in polytene chromosomes has been carried out in *Drosophilla*. In *Drosophila* system, a brief heat shock for 30-40 minutes induce puffs at several loci[2,3,5,6,14-16,24,25.] The salivary gland chromosomes of Chironomus also reveal several puffs in response to heat shock[17-21,26,30,31]

The existence of heat shock system in epidermal cells of foot pads of *sarcophaga bullata* & *pasasarcophaga ruficornis* has also been established. [7,8,12,23,31] Besides these, few other dipterans *ceratitis capitata*[9,22], *Lucilia*[11] and *Rhynchosciara*[29] show the stress response.

In dipterans, it has been shown that the specific puffs induced by heat shock could also be induces by recovery by anoxia and a variety of other agents some of which interfere with respiratory metabolism.[1,3,27,30]

In the present study, a cytological analysis of the response to chemical stress has been carried out in the foot pad polytene chromosomes of *Parasarcophag ruficornis.*

Material and Methods

For observing the normal and experimentally induced *(in vitro)* puffing activity, the foot pads of male pupae of *P. ruficornis* were dissected in insect saline. The pupal cuticle was removed and then food pads were incubated in sodium azide, 2-4 dinitrophenol & benzamide to study the stress response of polytene chromosomes. Control and heated food pads were fixed in acetoalcohal (1 part glacial acetic acid:3 parts ethanol, for 2 mint. and stained in 3% aceto-orcein for 15-20 min., squashed under a clean coverslip and sealed with DPX.

The chromosome regions & puffs were identified on the basis of standard cytological maps of *Parasarcophaga ruficornis.*[13,28] Puffing activity was determined as the ratio of diameter of the puffed region (D) with that of the neighbouring non-puffed region (d) in the chromosome as per the method.[4] In all experiments, the mean size of a puff has been calculated on measurements of 10 puffs.

Result and Discussion

In vitro treatment of foot pads with different chemicals namely sodium azide, 2-4dinitrophenol & benzamide, induced a single large puff in the chromosome IIL at region 12A in the polytene chromosomes of *P.ruficornis*. The puffing activity at all the other loci was however unaffected.

Chemical induced puffing

1. **Sodium Azide –** The foot pads of *Parasarcophaga ruficornis* were incubated at 28^0c in insect saline containing 2x10-2M sodium azide for 30,60,90,120,150 & 180 min. The control foot pads were incubated in sodium azide free insect saline. Incubation of foot pads

TABLE-1. Effect of Sodium azide on hs puff response in pupal foot pads of *P. ruficornis.*

Species		Puff size					
		30 min	60 min	90 min	120 min	150 min	180 min
P.ruficornis							
Control	Mean S.E.	1.55±0.04	1.60±0.04	1.55±0.05	1.50±0.06	_	_
Treated	Mean S.E.	1.78±0.07	2.45±0.05	2.50±0.06	3.78±0.23	3.33±0.15	3.12±0.25

of *P.ruficornis* in sodium azide ($2x10^{-2}$M in insect saline) at normal growth temperature (28^{r}C) results in the induction of hs puff without causing regression of puffs at others loci. The rate of induction is very slow and the maximum size of the puff is reached only after 120 min, thereafter the size remains more or less the same till 180 min. The mean size of puff in control and in sodium azide treated foot pads of *P. ruficornis* is presented in Table 01 and the puffs are illustrated in Fig. 01.

2. **2-4 Dinitro phenol (DNP) –** The pupal foot pads of *P. ruficornis* were incubated at 28^{0}C in insect saline containing 10^{-4} M 2-4 dinitro phenol (pH 5.8) for different time intervals , viz; 30,60,80,120,150 & 180 min. control foot pad were incubated in DNP free insect saline. Incubation of food pads of *P.ruficornis* with 2-4 dinitrophenol (10^{-4} M in insect saline, ph 5.8) results in the induction of hs puff within 60 min, without causing regression of any other puff. In controls the puff size remains unchanged during incubation in saline for the same time period. The induced hs puff maintains more or less similar size from 60 min onward till 180 min. The mean size of the hs puff in treated and control foot pads is presented in Table 2 and the induced puffs are illustrated in Fig 2.
3. **Benzamide –** The foot pads of *P.ruficornis* were incubated in 28^{0}C in insect saline 1mg/ml benzamide for different time intervals *viz;* 30, 60, 90, 120, 150 & 180 min. The control foot pads were incubated in benzamide free medium. Incubation of foot pads of *P. ruficornis* in benzamide (1mg/ml) results in the induction of hs puffs within 30 min and thereafter the activity of puff is more or less similar upto 180 min, without any appreciable change in size of the puff (Fig. 03). The size of the benzamide induced hs puffs is presented in Table 03.

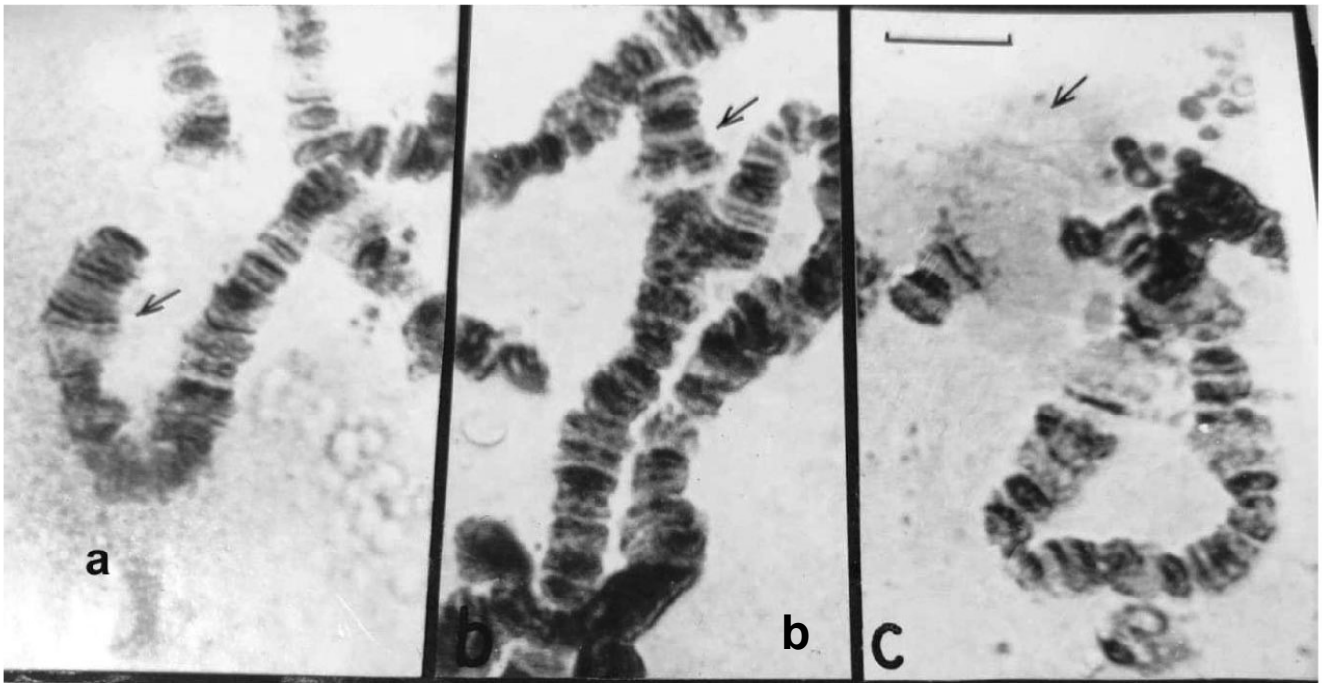

Fig.1 : Induction of hs puff by Sodium azide in *P.ruficornis* a-control, b-after 60 min,c-after 120min.

TABLE-2 : Effect of 2-4 dinitrophenol on hs puff response in pupal foot pads of *P. ruficornis*

Species		Puff size					
		30 min	60 min	90 min	120 min	150 min	180 min
P.ruficornis							
Control	Mean S.E.	1.79±0.05	1.78±0.05	*	*	*	*
Treated	MeanS.E.	2.10±0.05	3.50±0.09	3.40±0.09	3.44±0.06	3.33±0.21	3.50±0.13

* Due to a very small number of pupae available, controls for 90, 120, 150 and 180 min could not be kept.

TABLE-3 : Effect of benzamide on hs puffs response in pupal foot pads of *P.ruficornis.*

Species		Puff size					
		30 min	60 min	90 min	120 min	150 min	180 min
P.ruficornis							
Control	Mean S.E.	1.75±0.06	*	*	*	*	*
Treated	MeanS.E.	3.10±0.12	2.90±0.06	2.98±0.09	2.92±0.09	3.20±0.15	3.25±0.08

* Due to a very small number of pupae available, controls for 60,90, 120, 150 and 180 min could not be kept.

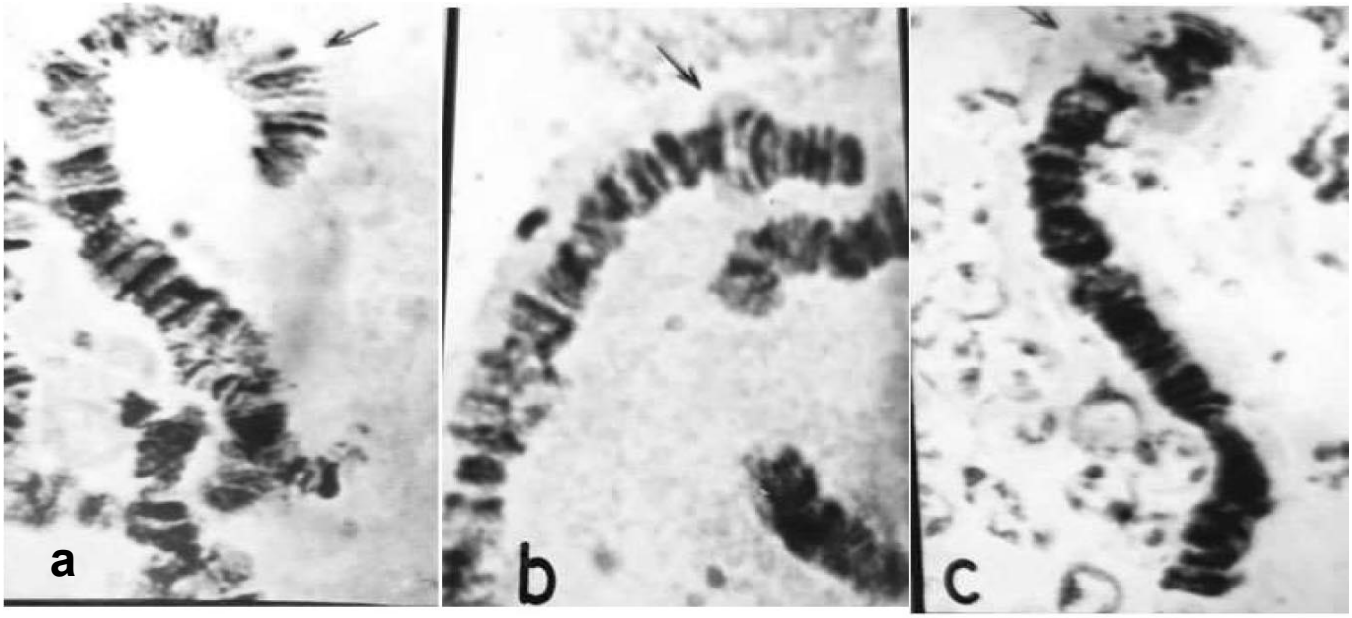

Fig. 2 : Induction of hs puff by 2-4- dinitrophenol in *P. ruficornis* a-control, b-after 30min,c-after 60min (arrow denotes hs puffs)

A single large puff is induced near the telomeric end of chromosome arm of IIL. The hs puff (heat shock) is also found in the same locus in other species of *Parasarcophaga*[12] and *Sarcophaga bullata*.[7,8] This puff is also induced by cold shock in *Sarcophaga*.[31] It seems, the puffing response to different stresses in Sarchophagids is due to a common set of gene loci. Thus this appears that single conserved prominent hs puff is a distinctive feature of the heat shock & chemical stress response in Sarcophagids.

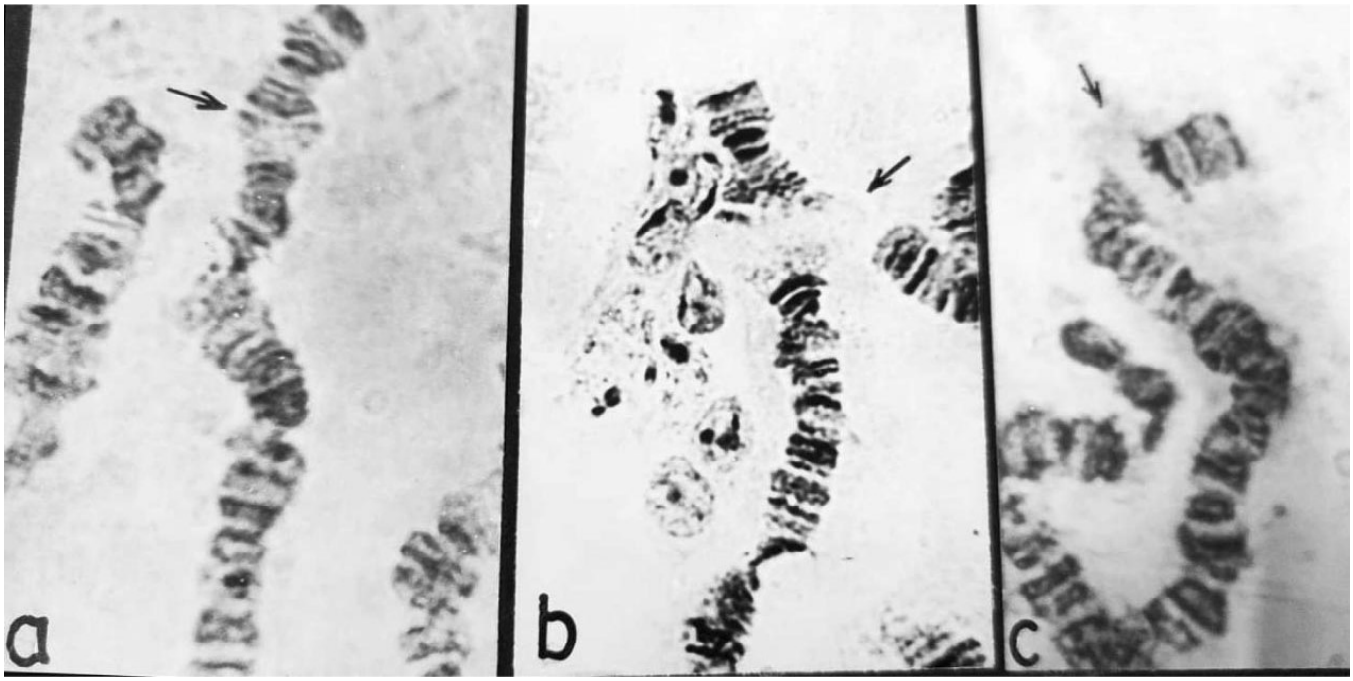

Fig. 3 : Induction fo hs puffs by benzamide in *P. ruficornis* a-conrol, b-after 30min, c-after 60min. (arrow denotes hs puff)

References

1. Akanksha singh, Rashmi singh, U.R. Agrawal, R.R. Tiwari. "Stress response of polytene chromosomes of *Sarcophaga ruficornis* (FAB.) (Sarcophagidae : Diptera) to lead nitrate. *Int. J. pharm Bio Sci.* 2012 july; **3** (3) : B 578-583.
2. Ashburner M.Patterns of puffing activity in the salivary gland choromosomes of *Drosophilla V*. Responses to envrironmental treatments. *Chromosoma.* 1970; **3** : 365-376.
3. Ashburner, Bonner J.J. The induction of gene activity in *Drosophilla* by heat shock. *Cell.* 1979; **17**:241-254.
4. Berendes HD. Salivary gland function and chromosomal puffing pattern in *Drosophila hydei*. *Chromosoma.* 1965; **17** : 35-77.
5. Berendes, Holt, T.K.H. The induction of chromosomal activities by temperature shock. *Genen Phaenen.* 1964; **9**: 1-7.
6. Berendes, Breugel, F.M.A. van, Holt, T.K.H. Experimental puffs in salivary gland chromosomes of *Drosophila hydei*. *Chromosoma.* 1965; **16**: 35-46.
7. Bultmann H. Heat shock responses in polytene foot pad cells of *Sarcophaga bullata*. *Chromosoma.* 1986a; **93**: 347-357.
8. Bultmann H. Induction of a heat shock puff by hypoxia in polytene foot pad chromosomes of *Sarcophaga bullata*. *Chromosoma.* 1986b; **93**:358-366.
9. Chrysanthakopoulou A, Mintzas AC, Zacharopoulou A. Heat shock puffs and genes in the Mediterranean fruit fly *Ceratitis capitata*. Proc. Fifth International Symposium on Fruit Flies of Economic Importance FAO/IAEA. Penang, Malaysia. 1998.
10. el Agoze, M. Puffing activity after heat shock in *Musca domestica*. *J. Egypt Soc. Parasitol.* 1993; **23**: 171-177.
11. Joshi A, Tiwari PK. Chromosomal responses of blowfly *Lucilia cuprina* to heat and heavy metal stress. *Genetica.* 2000; **109**: 211-218.
12. Kaul D, Ranjan A. Evolutionary conservation of heat shock puff in different species of Sarcophagidae. *Nucleus*. 1962; **35**: 52-54.
13. Kaul Agrawal, UR, Tewari RR. Puffing activity in the foot pal chromosomes *of Parasarcophaga ruficornis* (Fab)(*Sarcophagidae : Diptera*). La Kromosomo II. 1983; **32** : 961-970.

14. Lakhotia SC, Prasanth KV. Tissue and development specific induction and turnover of hsp 70 transcripts from 87A and 87C loci after heat shock and during recovery in *Drosophila melanogaster*. *J. Exp. Biol.* 2002; **205**: 345-358.
15. Lakhotia SC, Singh AK. Conservation of the 93D puff of *Drosophila melanogaster* in different species of *Drosophila*. *Chromosoma.* 1982; **86**: 265-278.
16. Lakhotia SC, Singh AK.Non-Inducibility of the 93D heat shock puff in cold reared larvae of *Drosophila melanogaster*. *Choromosoma.* 1985; **92**: 45-54.
17. Lezzi M. Heat shock phenomenon in *Chironomus tentans*. II. *In vitro* effects of heat shock and over heat on puffing and their reversal. *Chromosoma.* 1984; **90**: 198-203.
18. Lezzi M, Meyer B, Mahr R. Heat shock phenomenon in *Chironomus tentans*. I. *In vivo* effects of heat , over heat and quenching on salivary chromosome puffing. *Choromosoma.* 1981; **83**: 327-339.
19. Morcillo G, Barettino D, Diez JL. Heat shock puffs in isolated salivary glands of *Chironomus thummi. Bio. Cell.* 1982; **44**: 221-227.
20. Morcillo G, Diez JL, Botella LM. Heat shock activation of telomeric sequences in different tissues of Chironomus thummi. *Exp. Cell Res.* 1994; **211**: 163-167.
21. Nath BB, Lakhotia SC. Heat shock response in a tropical *Chironomus:* seasonal variation in sesponse and the effect of developmental stage and tissue type on heat shock protein synthesis. *Genome.* 1989; **32**: 676-686.
22. Papadimitriou E, Kritikau D, Mavroidis M, Zacharopoulou A, Mintzas AC. The heat shock 70 gene family in the Mediterranean fruit fly *Ceratitis capitata*. *Insect Mol. Biol.* 1998; **7**: 279-290.
23. Ranjan A, Kaul D. A heat shock induced puff in foot pad cells of *Parasarcophaga ruficormis* (Fab.) (*Sarcophagidae : Diptera*). *CIS (Japan).* 1988; **44**:5-6.
24. Ritossa F. Attivita sintetiche al livello dei puffs in *Drosophila busckii*. *Atti. Ass. Genet. Ital.* 1962; **7**: 147-156.
25. Ritossa F. Experimental activation of specific loci in polytene chromosomes of *Drosophila*. *Exp. Cell Res.* 1964; **35**: 601-607.
26. Saas H. Transcription of heat shock gene loci versus non-heat shock loci in *Chironomus* polytene chromosomes : evidence for heat induced formation of novel putative ribonucleo-protein particles (hn RNPs) in the major heat shock puffs. *Chromosoma.* 1995; **103**: 528-538.
27. Schlesinger MJ, Ashburner M, Tissieres A. Heat Shock from Bacteria to mam. Cold Sping Harbor Lab, Cold Spring Harbor, New York. 1982.
28. Shrivastava US, Shukla KM, Kaul D, Tewari RR. The polytene chromosomes of *Parasarcophaga ruficornis* (Fab)(Sarcophagidae:Diptera). *Proc. Nat. Acad. Sci. (India).* 1982; **52**(B): 155-164.
29. Stocker AJ, Madalena CRG, Gorab E. The effects of temperature shock on transcription and replication in *Rhynchosciara Americana* (Diptera : Sciaridae). *Genetica.* 2006; **126**: 277-290.
30. Tripathi J, Agrawal UR, Tiwari RR. Chromosomal response to chemical stress in flesh fly *Sarcophaga ruficornis* (Fab.) (Sarcophagidae : Diptera) *Cytologia.* 2009; **74**(2) : 177-183.
31. Tripathi J, Agrawal UR, Tiwari RR. Choromosomal response to cold shock in fleshfly *Sarcophaga ruficornis* (Fab) (Sarcophagidae : Diptera). *Int J. Pharm Biosci.* 2013; **4**(1) : (B) 1057-1061.