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GENOTOXIC EFFECTS OF CADMIUM CHLORIDE ON POLYTENE CHROMOSOMES OF *SARCOPHAGA RUFICORNIS* (FAB.) (SARCOPHAGIDAE: DIPTERA)

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ABSTRACT

Cadmium ranks close to lead and mercury in toxicological importance due to its increasing levels in the environment, as a result of industrial practices of past and present. Chromosomal responses to cadmium chloride shocks were studied in the pupal footpad polytene chromosomes of *Sarcophaga ruficornis*. Cadmium chloride induces a single puff on chromosome arm IIL at the region 12A by *in vitro* treatment. Heat shock and other chemical stresses is also induced similar puff in several sarcophagids. Thus a single prominent puff is a hallmark of stress response in sarcophagids, so they are proposed as a tool providing early warning of adverse long term effects of toxic agents on the genome.

INTRODUCTION

Sarcophaga ruficornis is an abundant, well known flesh fly species of medical importance both as a myiasis-producing agent and fly seen in a forensic entomology context. Both natural and laboratory populations of this species have been extensively studied. For assessing the environmental pollutant, *Sarcophaga ruficornis* was the first species among sarcophagidae used to cytologically analyze foot pad polytene chromosomes of pupa collected from laboratory stock with substrate containing a heavy metal i.e. cadmium chloride. Genotoxic effect of cadmium chloride may result from a variety of factors, including non specific effects at very high doses of cadmium chloride exposure. Pupa of *Sarcophaga ruficornis* which was 7 day old, the polytene chromosome of foot pads are known to have DNA strands replicated, and doubling of this pre-existing amount which result from more than a thousand such replications (Whitten, 1969). This feature allows us to observe the mechanisms of the stress responses and the function of heat shock genes in response to thermotolerance and adaptation to chemical stresses (Bultman, 1986a, b; Lezzi, 1984; Lezzi *et al*., 1981; Nath and Lakhotia, 1989; Singh, *et al*., 2012; Singh and Singh, 2015; Vincent and Tanguay, 1979; Yamamoto, 1970).

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In the present study, a cytological analysis of the chemical i.e. cadmium chloride induced chromosomal puff was made to identify the genetic loci responsive to these stress factors. Pupa of *Sarcophaga ruficornis* have been used for toxicity testing because they are widely distributed, highly responsive to environmental stress and represent the developmental stage in the life cycle of species.

MATERIALS AND METHODS

The stock of *Sarcophaga ruficornis* used in this experiment originated from gravid female or a single copulating pair collected from wild and maintained at 27±1ºC following the method of Singh *et al* 2012. Sarcophagid pupae possess excellent foot pad chromosomes with a distinct and well characterized band structure that allows precise cytogenetic analysis (Kaul *et al*., 1983; Ranjan and Kaul, 1988) and follow up of the effects of contaminants like heavy metal on the puffing activity of polytene chromosomes. Moreover, the standard karyological characteristics of some species (Kaul *et al*., 1983; Kaul and Ranjan, 1992; Ranjan and Kaul, 1988) can be employed as a basis to reveal the changes in effect of heat shock treatment by studying changes in puffing activity of the polytene chromosomes.

Cadmium chloride as important Contaminants

It required as trace elements for normal cellular functions. Their presence in trace amounts is considered to be important for living organisms, because they represent active sites for a number of enzymes which are involved in oxidation-reduction reactions. However, when their quantity exceeds the physiological concentration then it is toxic to cells. Effects of cadmium chloride can be deleterious when foot pads were exposed to a particular concentration for certain time period. As a consequence, it has been lead to changes in puffing activity of polytene chromosomes.

Heavy metal treatment and induction of Chromosomal puffs

For each treatment only male pupae that developed up to the phase of 7 day were dissected in insect saline. Foot pads were incubated in cavity block at different concentrations such as 10 mM, 20 mM, 30 mM and 40 mM for 10 min, 20 min, 30 min, 40 min, 50 min, 60 min, 70 min, 80 min, 90 min and 100 min. at room temperature (27±1ºC). Control and Cadmium chloride treated foot pads were immediately fixed in aceto alcohol (1 part glacial acetic acid: 3 part ethanol) for 2 min. After fixation, the foot pads were stained with 2% lacto-aceto-orcein for 40-45 min. and stained foot pads were squashed in a fresh drop of acetic acid under a clean coverslip. Coverslip was sealed from the sides with nail polish to prevent from drying and stored at 4 ºC.

Puff size measurement

The temporary squash preparation of control and induced puffs were measured by the occulomicrometer scale using a ratio of diameter of puffed region (D) with that of the neighboring non puffed region (d) on the chromosome arm (Berendes, 1965). In each case a minimum of 20 puffs with well spread chromosomes were selected for the measurement of puff sizes (D/d). By the standard cytological maps of *Parasarcophaga ruficornis*, chromosomes regions and puffs were identified (Srivastava *et al*., 1982; Kaul *et al*., 1983). Photographs of the chromosomes were taken using a Nikon Eclipse 80i, microscope equipped with CCD Camera (ACT-1 Software).

RESULTS

Sarcophaga ruficornis has 6 chromosomes (2n). The species was identified karyotypically. For the study of stress- induced puffs, foot pad of male pupa were used to making the polytene chromosome. When foot pad of the 7 day old male pupa were exposed to 10 mM concentration of cadmium chloride for different time interval which was 10 min, 20 min, 30 min, 40 min. 50 min, 60 min, 70 min, 80 min, 90 min and 100 min resulting into no puffing response in any chromosome arm against this treatment. The treatment of foot pads with 20 mM, 30 mM and 40 mM concentrations of cadmium chloride showed the puffing activity in chromosome arm from 20 min to 100 min time interval.

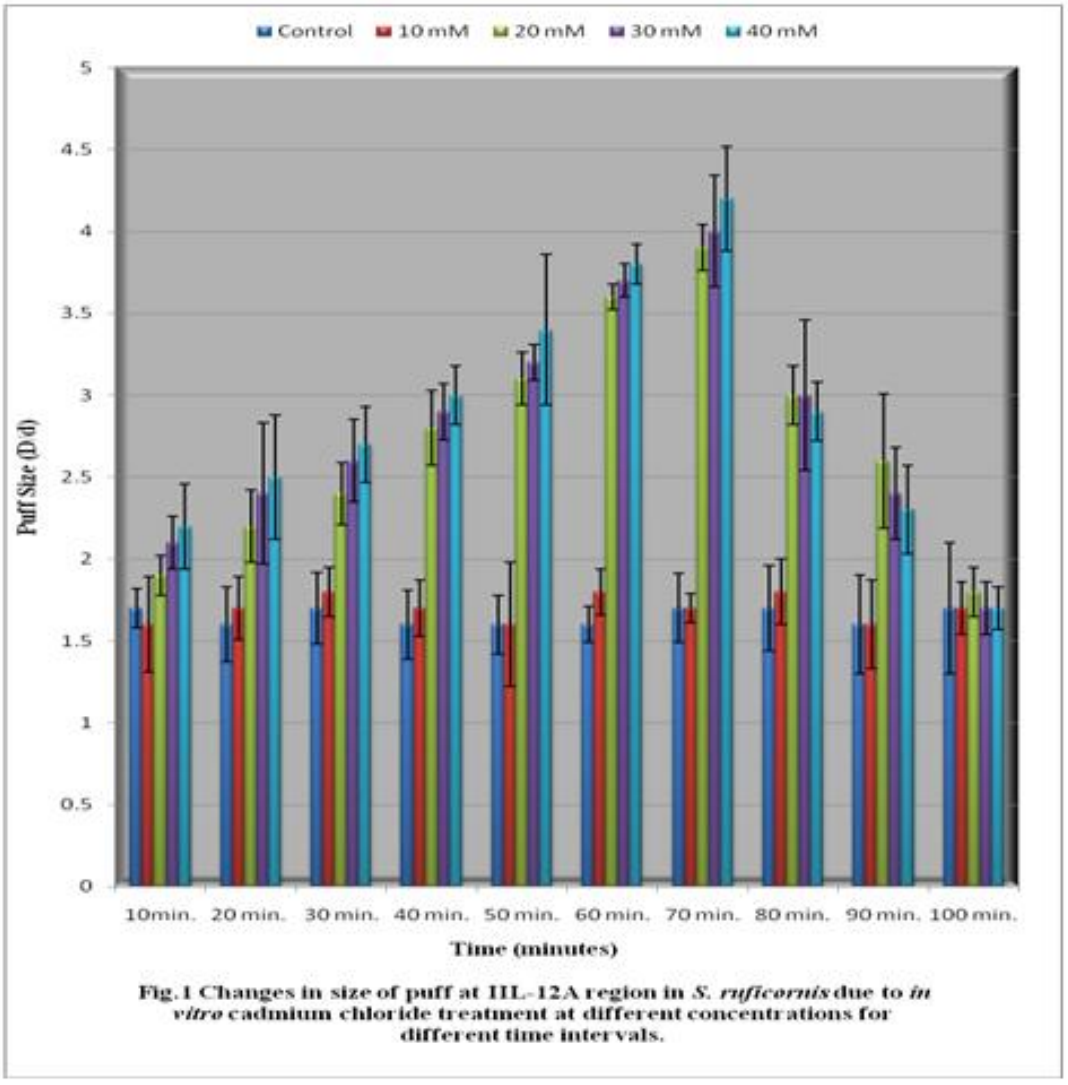

Fig.1 Changes in size of puff at IIL-12A region in *S. ruficornis* due to *in vitro* cadmium chloride treatment at different concentrations for different time intervals.

But puffing response was not shown in 10 min of incubation for all concentration. There was a single puff which have induced in chromosome arm IIL at 12A region whereas, there was no changes in puffing activity of other chromosome arm as well as other loci.

The puffing activity was maximally induced at 70 min time of incubation in the three concentrations i.e. 20 mM, 30 mM and 40 mM. The puff size increases gradually, and attained maximum size thereafter puffing activity was to be continuously decreases.

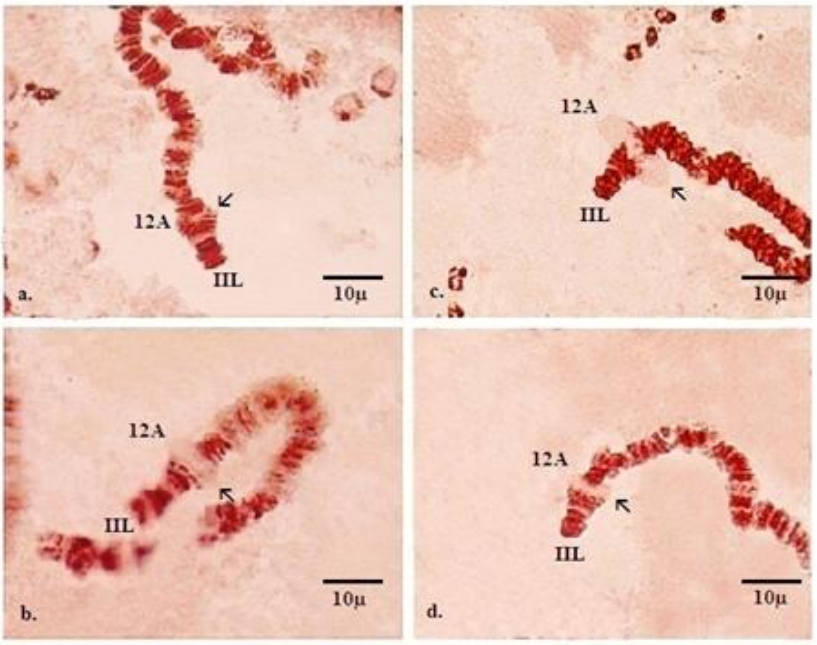

Fig. 2. Puff induction at chromosome region IIL-12A of *S. ruficornis*

a. Control
b. *in vitro* treatment with cadmium chloride at 20 mM for 10 min
c. 20 mM for 70 min.
d. 20 mM for 100 min.

Bar represents 10µ.

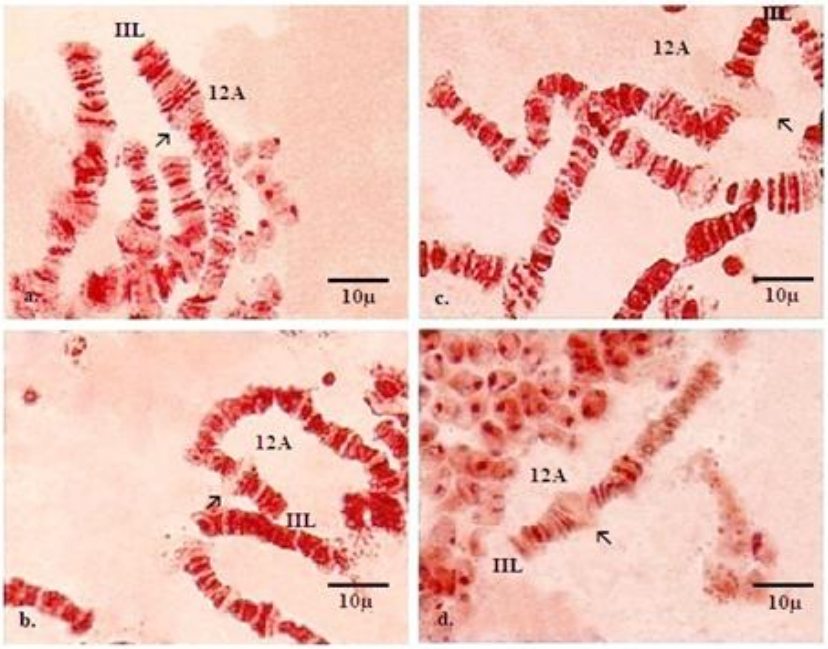

Fig. 3 . Puff induction at chromosome region IIL-12A of *S. ruficornis*

a. Control
b. *in vitro* treatment with cadmium chloride at 30 mM for 10 min.
c. 30 mM for 70 min.
d. 30 mM for 100 min.

Bar represents 10µ.

At 100 min the activity of puff size is very low i.e. completely regressed (Fig. 2-4). The correlation of puff size with activity of a particular locus is to be known. There was three distinct changes were observed with cadmium chloride treatment along with chromosomes, i.e. induction, regression of puffing activity and inhibition of either puff activation or puff regression.

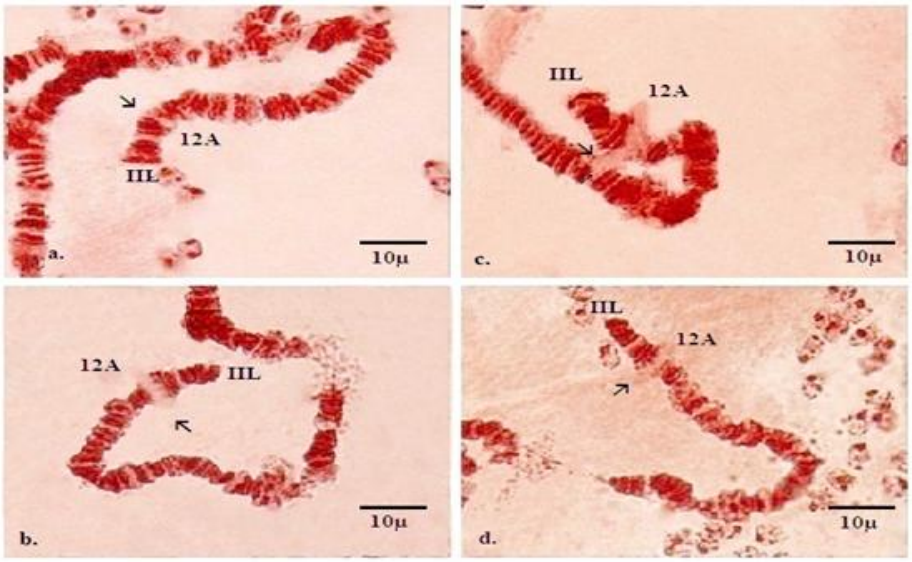

Fig. 4 . Puff induction at chromosome region IIL-12A of *S. ruficornis*
a. Control
b. *in vitro* treatment with cadmium chloride at 40 mM for 10 min.
c. 40 mM for 70 min.
d. 40 mM for 100 min.

Bar represents 10µ.

The mean size of puff in treated and control foot pads are showed in graphical presentation (Fig. 1). In all condition, gene activity has to be change with cadmium chloride, relative to untreated pupa is presented in Fig. 2, 3 and 4. The ability of chemicals to activate stress puff has shown to be dependent on the concentration and time variation of cadmium chloride treatment used in *Sarcophaga ruficornis.* These are shown to by increase of expression of protein (Hsp) for certain time and Hsp decreases gradually with increasing time for the stresses. Maximum decrease was observed in puffing activity at 90 min which were explained as sublethal concentration of cadmium chloride treatment which are dependent on the time interval. (Fig.1) Sublethal concentration was to be maximally found at 90 min for 40 mM and then for 30 mM concentration of cadmium chloride. However, sublethal concentration of 20 mM was also found at 90 min, but its effect was relatively lower than that of 30 mM and 40 mM (Fig.1). Significant increase and decrease of puff size is considered to be dose and time depended which is symptom of stress evoked by cadmium chloride treatment.

DISCUSSION

The response of *Sarcophaga ruficornis* genome to cadmium chloride is characterized by gene expression. Alterations in gene expression are indicated by changes in puffing activity to the chemical stress. Such changes in puff activity also occur following exposure to the other stressors i.e. Lead nitrate and Chromium nitrate (trace metals) (Singh *et al* 2012; Singh and Singh 2015). After the treatment of footpad with Cadmium chloride, a single puff which is very prominent puff is induced in the chromosome arm IIL at the region 12A. Initiation of puffing activity shows that the puff induction starts at particular time for all concentration except 10 mM, the initiation time was 20 min. for three concentrations i.e. 20 mM, 30 mM and 40 mM. However, the initiation of puffing activity fails at 10 min time of incubation at all concentration. In general, puff size is known to correlate with activity of a particular locus. In each concentration of cadmium chloride, the puffing activity is to be increasing gradually and attain a maximum size i.e. the large size of the puffs which would suggest that the decondensation of the polytene chromosome is likely occurring over a fairy large area (Winegarden *et al*., 1996). Thereafter, the level of puffing activity is to be decreasing gradually and at the end it is completely regressed. It is important to known that, when the chromosome was incubated in chemical solution for longer time at all concentration except 10 mM, the puffing activity was to be slow down gradually and completely diminished at 100 min. As a consequence the chromosomes were not responding against the particular dose of chemical at 100 min because it becomes lethal to the cell.

When a cell experiences severe environmental stress, it stops or at least slows down most of its original functions such as transport process, DNA, RNA and protein synthesis. This is to understand that, the ability of chemicals to activate stress puff appears to be dependent on the concentration and time of incubation. Thus it can be hypothesized that the puffing activity reacts in the same way (i.e. with an induction and regression of puffing activity) to different concentration for different time of incubation of cadmium chloride. Induction of puffs by heat has been correlated with concomittant synthesis

of hsps, which apparently assist in the development of thermotolerance in *Drosophila* (Lindquist, 1986; Peterson, 1990; Ritossa, 1964; Spardling *et al*., 1977; Tissieres *et al*., 1974; Vincent and Tanguay, 1979) and *Chironomus* (Lezzi *et al*., 1981; Nath and Lakhotia, 1989). There was a single puff 12A on the left arm of chromosome IIL. No stress puffs were appeared on the other loci of chromosome. It is surprising that multiple cytological loci that are distinctly induced upon heat stress in *Drosophila* and *Chironomus*, while only one genetic locus showed significant inducibility in pupal foot pad polytene chromosomes of *Sarcophaga bullata* upon exposure to both heat and hypoxia. (Bultman, 1986a and b). Similar findings were also observed in *Sarcophaga ruficornis* (Singh *et al*., 2012; Singh and Singh, 2015) and *Lucillia cuprina* (Joshi and Tiwari, 2000).

Conclusion

The puffing activity was also observed in normal course of development, but it occurred at a very low frequency which strongly suggests that this is a random phenomenon and is not due to environmental stress. The reduction in puffing activity may be used as a biomarker of exposure to genotoxic substances inhibiting RNA synthesis. However, retainment of puffing activity of their high state of activity in *Sarcophaga ruficornis* suggesting that their function is not disturbed by contaminants. It is therefore unlikely that the appearance of this puff is connected to the synthesis of proteins, important for their survival in pollutant environments.

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