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FRAGMENTATION OF POLYTENE CHROMOSOMES OF *CHIRONOMUS STRIATIPENNIS* (KIEFFER) AS A MARK OF HEAVY METAL TOXICITY IN AQUATIC HABITATS

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ABSTRACT
The adults of Chironomids are though terrestrial, but in immature stages they pass their days in water. In aquatic bodies the flies at their larval stages live about 18-20 days and any incompatible situation in the habitats affects growth and development of the larvae. A study on the polytene chromosomes of the salivary gland cells of the larvae *of C. striatipennis* indicated that high level of toxicity due to heavy metals in the habitats led to fragmentation of their polytene chromosomes. Culturing of the larvae of this species in the laboratory with 30 mg/Kg of Cd with soil in the culture bed exhibited fragmentation of their polytene chromosomes in certain cases. In this toxicity impact the fourth polytene chromosome appeared to be more affected. Hence, a variation of response of different chromosomes of the fly to toxicity may be suggested.

INTRODUCTION

Chironomids are considered as good bioindicator for the environmental pollution (Vermeulen, 1995) and the larvae of the flies are mainly used as indicator of pollution in the aquatic medium because they live in aquatic body for more than 18 days (Bhaduri *et al.,* 2012). None of the other life stages are passed in water for so long period. Deformities as appear in different larval body parts of the larvae have been studied to estimate the effect of pollution in aquatic bodies (Bhattacharya *et al.,* 2005, 2006; Vermeulen, 1995). However, as impact of pollution many investigators have looked into organization of the polytene chromosomes developed in the larval body cells (Michailova *et al.,* 2001; Sella, 2006). In such investigations polymorphism of the polytene chromosomes could be noticed in several species of Chironimids. We have also noticed occurrence of polymorphism of polytene chromosomes in several species of Chironomus. The laboratory experiments involving the larvae of the species *C. striatipennis* to grow under artificially made polluted condition not only developed chromosomal aberrations but also fragmentation of the polytene chromosomes at higher dose. Hence, a correlation between pollution and polymorphic organization of polytene chromosomes may be suggested.

MATERIALS AND METHOD

Fourth instar larvae of *Chironomus striatipennis* Kieffer collected from heavy metal polluted aquatic bodies of Dhapa area of Kolkata were used in this investigation. Besides, the egg masses of the species of this *Chironomus* were cultured in the laboratory to develop 4th instar larvae for the present study. The larvae from the pure culture of the species were exposed to $CdCl_2$ at different concentrations as 1mg, 2mg, 3mg, 5mg, 10mg, 15mg, 20mg, 25mg and 30mg/ per Kg of soil in the culture beds. Fourth instar larvae developed in such cultures were taken for the present investigation.

The salivary glands from the fourth instar larvae collected from the natural habitats of the selected zone as well as from the laboratory cultures innoculated with different doses of cadmium salt were taken to prepare polytene chromosomes from them. The glands dissectedont over glass slides were fixed with aceto-alcohol (1:3) for 2-3 minutes and then stained with 2% aceto-carmine for a period of about 15 minutes. The stained tissue over the slide was then applied with 45% acetic acid for 5 minutes before squashing the tissue materials. Cover slip was applied over the tissue and the slide was taken within the folds of blotting paper for squashing. With thumb pressure the salivary gland tissues were squashed and the slides prepared thus were studied under the microscope for the study of the polytene chromosomes.

Along with this, the gland tissues were also squashed following fixation without staining. The cover glass over the squashed tissue was removed carefully so that squashed materials were not distorted. The slides with unstained squashed tissue materials was then treated with ethidium bromide solution (1μL in 10mL of phosphate buffer pH 7.2) for 2 minutes and

then the slide was rinsed in distilled water. After this the squashed materials were observed for incorporation of ethidium bromide in the tissue components under fluorescence microscope.

The soil sediments comprising the habitat of the Chironomus larvae and water samples Colleeted from the habitat were analyzed for the presence of heavy metals as As, Cd, Cu, Pb and Hg with the help of Atomic Absorption Spectrophotometer.

RESULTS

The salivary gland cells contained four polytene chromosomes and the chromosomes in their normal configuration displayed distinct bands, interbands, puffs and Balbiani rings (Fig. 1). The chromosomes were designated as I, II, III and IV according to their decreasing order of length. Out of these four polytene chromosomes the 4th polytene chromosome appeared to be more active having less heterochromatic bands and containing three Balbiani rings named as BR1, BR2 and BR3. Ethidium bromide treatment showed that ethidium bromide was incorporated into the chromosomes and they showed prominent fluorescence under Fluorescence Microscope (Fig. 4). Polytene chromosomes obtained from the larvae of polluted habitat showed polymorphism of all the polytene chromosomes and polymorphic forms included heterozygous inversion, deficiency and asynapsis (Fig. 2). The larvae also developed polymorphic polytene chromosomes raised under artificially developed polluted condition with cadmium in the laboratory. However, at the lower doses of cadmium in the culture media (1mg -10mg/Kg of soil in the bed) The Larval did not develop much polymorphism of the polytene chromosomes. On the other hand polymorphic chromosomes appeared at higher dose levels (*i.e.,* above 10mg of $CdCl_2$ per Kg of soil). It is noteworthy to mention here that at higher dose of cadmium in the culture medium (30mg/Kg of soil) not only developed aberrant polytene chromosomes but also showed fragmentation of polytene chromosomes (Fig. 3 and 4). This fragmentation of chromosome was prominent in the 4th polytene chromosome of the fly (Fig. 4 a and b).

The fragmented chromosome pieces in ethidium bromide treatment incorporated EtBr and therefore, they appeared as fluorescent fragments from polytene chromosomes (Fig. 4).

Result of heavy metal tests indicated that especially the soil sediments of aquatic bodies at Dhapa contained high amount of heavy metals compared to water samples (Table 1).

Table 1: Heavy metal concentration in aquatic bodies of Dhapa region of Kolkata

Sample	As(mg/L)	Cd(mg/L)	Cu(mg/L)	Pb(mg/L)	Hg(mg/L)
Soil	2.04	1.37	101.25	157.36	0.64
Water	<.005	<.005	<.05	<.002	<.001

DISCUSSION

Presence of four polytene chromosomes in the salivary gland cell is the universal occurrence in most of the Chironomid larvae and these appear to be the cytological expression of eight chromosomes as observed in a species with eight diploid numbers of chromosomes. These polytene chromosomes are designated as I, II, III and IV according to their decreasing order of lengths (Devai *et al.,* 1989, Keyl, 1962). The acrocentric smallest fourth polytene chromosome in *C. striatipennis* exhibited many altered forms but the distorted 4th chromosome with many fragments/appeared to be the optimal cytological expression of the 4th chromosome.

The unique configuration of the polytene chromosomes with their exhibition of bands, interband and puffs appears to be Nature's collective effort on gene expression at the cytological level and in the living world the dipterans display this type of unique chromosomes. Many of the scientists in the field of Molecular Biology and Genetics have preferred the polytene chromosomes of dipterans as the object for study for revealing many tricks in genetic phenomena in the living world (Berezikov *et al.,* 1998; Case and Daneholt, 1978, Michailova, 2011). The present investigation has revealed the interaction of genes and environment when the expression is displayed through the polytene chromosomes of the flies. The chromosomes in the cell are the vehicles of genes in which the genetic materials remain as a dormant pith hardly detectable at the visibility unless realizable by way of some phenotypic expression. The polytene chromosomes in *C. striatipennis* appeared as elongated filamentous bodies with the display of many bands and interbands alternately along each of the arms of the chromosomes. Displayed puffs and Balbiani rings at some regions of the chromosome arms appeared as the sites of gene activity showing unusual texture of the genetic material at specific locations. The appearance of several aberrant morphology of the polytene chromosomes in this fly collected both from nature and the laboratory cultures in artificial condition represented some unusual expression of the genetic material The appearance of the aberrant forms of polytene chromosomes in the larvae of the Chironomids has also been observed by many investigators throughout the globe (Michailova *et al.,* 2002, Todorova, 2000). However, the investigators advocated that the environmental pollution due to heavy metals might be one of the multiple reasons behind the causation of structural variations and aberration of the polytene chromosomes (Michailova and Belcheva, 1990). It was found by some investigators that there was a dose dependent response of the polytene chromosomes to the occurrence of active puffs and lead concentration (Michailova *et al.,* 2002) appeared to be related with a number of structural aberrations. The induction test with Cd mixed in the culture tray with soil in the bed appeared to be highly effective in causation of polytene chromosome polymorphism and the observation is supportive to findings of other investigators on the polytene chromosome polymorphism and aquatic toxicity (Michailova *et al.,* 2006).

The ISI standard for irrigation water is 0.01 mg/L for Cd. Hence the concentration of Cd as found in the natural habitats of Dhapa was quite high for smooth development of the larvae of *C. striatipennis*. For some other heavy metals also the concentrations of the toxic heavy metals were high in the sampling sites as ISI standards for some of the heavy metals were reported as 0.05 mg/L for Cu, 0.001 mg/L for As, 0.006mg/L for Hg and 10 μg/L for Lead. However, the population of the *Chironomus* larvae was quite high and the population of the larvae was not low even at this toxic condition. This indicated that the growth of the Chironomids could not be affected by

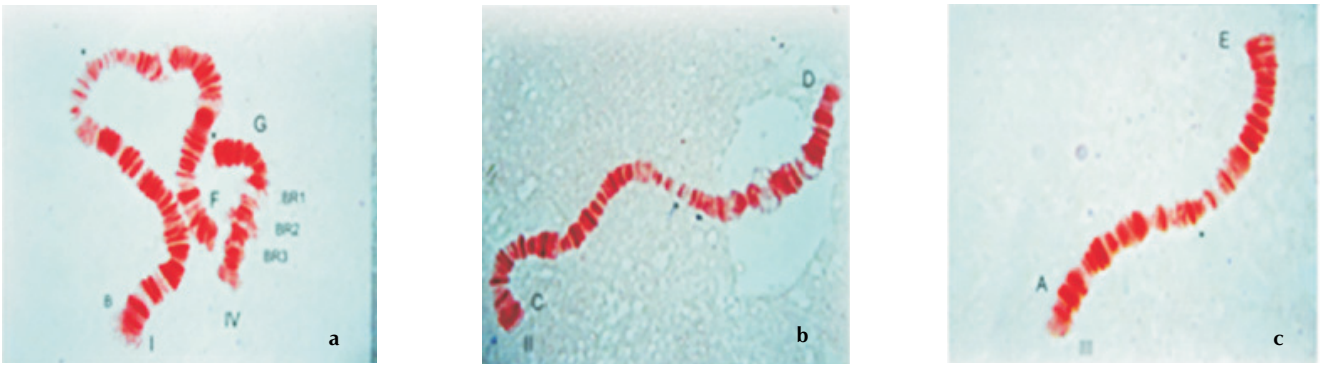

Figure 1: Four polytene chromosomes of *C. striatipennis*. The chromosomes are marked as I, II, III and IV according to decreasing order of lengths. A to G represent arm combinations in the chromosomes. Centromere in the chromosome is marked with * and BR denotes the Balviani ring

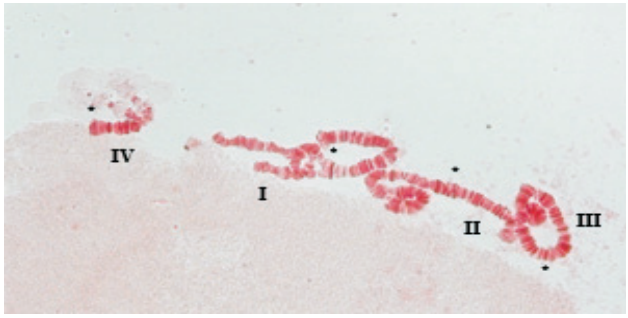

Figure 2: Natural polymorphism of the polytene chromosomes of *C. striatipennis;* I, II III and IV represents four polytene chromosomes. Asynapsis, deficiency and inversion are involved in causation of polymorphism of the polytene chromosomes

the presence of the pollutants in the habitats; rather the Chironomid larvae appeared to be highly tolerant to the levels of pollution. This on the other hand advocates the plasticity of the Chironomid tolerance to different degrees of pollution. This phenomenon of plasticity in tolerance to pollution by Chironomids suggests the presence of some genetic phenomenon in Chironomids to combat pollution by physiological mechanism. Even at the condition when polytene chromosomes get fragmented, the development is not at all affected and the larvae can complete their development producing the adults at the last. Hence, fragmentation of the polytene chromosomes appears to be a local effect on the polytene chromosomes without affecting differentiation at the other body parts of the larva.

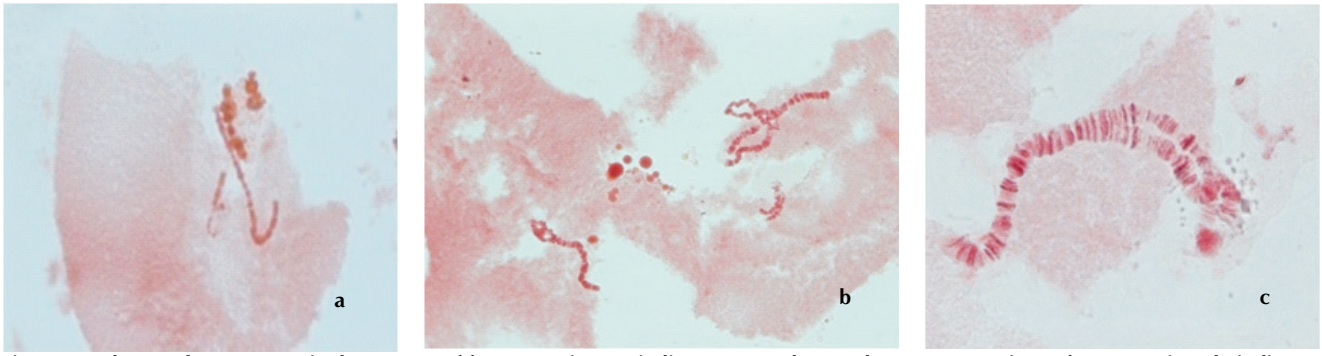

Figure 3: Polytene chromosomes in the process of fragmentation. (a) indicates two polytene chromosomes prior to fragmentation, (b) indicates fragments of chromosomes in the midst o the polytene chromosomes and (c) aberrant third polytene chromosome with small fragments of chromatin at the terminal part of the chromosome

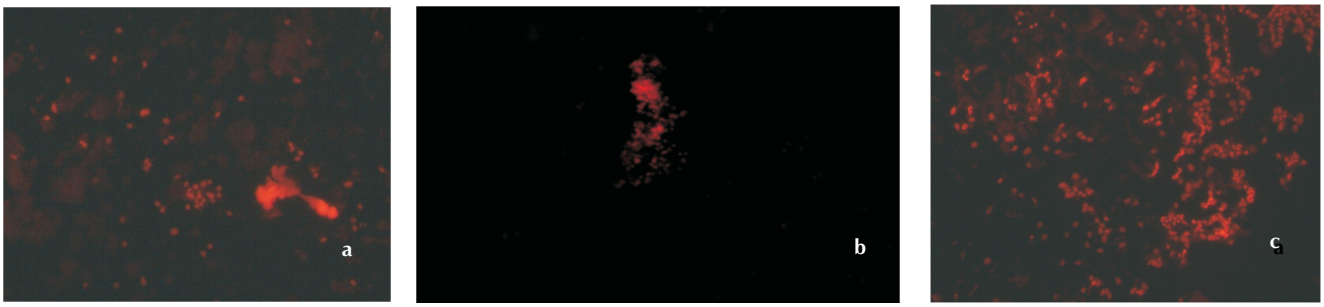

Figure 4: Fragmentation of chromosome. (a) and (b) show the fragmentation of 4th polytene chromosome and (c) shows dispersed chromosome fragments. EtBr incorporation makes the chromosome fragments fluorescent

Further, heavy metal toxicity may affect the polytene chromosomes in such a way that they may be fragmented under the influence of heavy metals. Thus, the naturally obtained fragmentation of polytene chromosomes appears to be indication of high concentration of heavy metals in the habitat. Because the laboratory induction test also produced fragmented polytene chromosomes in the salivary gland cells. Therefore, different nature of polymorphism of polytene chromosomes of *C. striatipennis* Kieffer may be indicative of level of heavy metal pollution in the aquatic habitats.

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REFERENCES

Berezikov, E., Blinov, A. G., Scherbik, S., Cox, C. K. and Cases, S. T. 1998. Structure and polymorphism of the *Chironomus thummi* gene encoding special lobe-specific silk protein, hsp 160. *Gene*. **223:** 347-354.

Bhaduri, S., Sarkar, P., Ghosh, C. and Midya, T. 2012. Laboratory rearing of *Chironomus striatipennis* Kieffer (Diptera: Chironomidae). *Proc. Zool. Soc.* **65(2):** 121-125.

Bhattacharya, G., Sadhu, A. K., Mazumdar, A. and Chaudhuri, P. K. 2005. Antennal deformities of chironomid larvae and their use in biomonitoring of heavy metal pollution in the River Damodar of West Bengal, India. *Environ. Monit. Assess*. **108:** 67-84.

Bhattacharya, G., Sadhu, A. K., Mazumdar, A., Mazumdar, U., Chaudhuri, P. K. and Ali, A. 2006. Assesment of impact of heavy metals on the communities and morphological deformities of Chironomidae larvae in the River Damodar (India, West Bengal). *Supplementa ad Acta Hydrobiologica*. **8:** 21-32.

Case, S. T. and Daneholt, B. 1978. The size of the transcription unit in Balbiani Ring 2 of *Chironomus tentans* as derived from analysis of the primary transcript and 75 S RNA. *J. Molecular Biology*. **124:** 223-241.

Devai, G., Miskolezi, M. and Wulker, W. 1989. Standardization of chromosome arm B, C and D in *Chironomus* (Diptera, Chironomidae). *Acta Biologica Debrecina Supplementum Oecologia Hungarica*. **2:** 79-92.

Keyl, H. G. 1962. Chromosomenevolution bei *Chironomus* II. Chromosomenumbauten und phylogenetiische Beziehungen der Arten. *Chromosoma (Berlin.)* **13:** 464-514.

Michailova, P. 2011. Rearrangements in Chironomidae (Diptera) Genomes induced by various environmental stress factors, Russian *J. Genetics, Applied Research*. **1(1):** 10-20.

Michailova, P., Petrova, N., Ilkova, J., Bovero, S., Brunetti, S., White, K. *et al.* 2006. Genotoxic effect of copper on salivary gland polytene chromosomes of *Chironomus riparius* Meigen 1804. (Diptera, Chironomidae). *Environmental Pollution*. **144:** 647-654.

Michailova, P. and Belcheva,R. 1990. Different effects of lead on experimental morphology and polytene chromosomes of *Glyptotendipes barbiceps* (Staeger) (Diptera: Chironomidae). *Folia Biologica*. **38:** 83-88.

Michailova, P., Todorova, K. and White, K. 2002. The Effect of Lead on the Salivary Gland Chromosomes of *Glyptotendipes salinus* Michailova (Chironomidae, Diptera), Biologia, Section, Cellular and *Mol. Biol.* **57(3):** 359-367.

Michailova, P., Petrova, N., Sella, G., Bovero, S., Ramella, L., Regoli, F. and Zelano, V. 2001. Genotoxic effects of chromium on polytene chromosomes of *Chironomus riparius* Meigen 1804 (Diptera Chironomidae). *Caryologia*. **54:** 59-71.

Sarkar, P., Bhaduri, S., Ghosh, C. and Midya, T. 2011. A study on the polymorphic fourth chromosome of *Chironomus striatipennis* (Kieffer). *The Bioscan*. **6(3):** 383-387.

Sella, G. 2006. Genotoxic effect of copper on salivary gland polytene chromosomes of *Chironomus riparius* Meigen 1804 (Diptera, chironomidae). *Environmental Pollution*. **144:** 647-654.

Todorova, J. 2000. Cytotaxonomic variability of *Chironomus riparius* Meigen (Diptera, Chironomidae) from anthropogenically influenced regions in Bulgaria. *Acta. Zool. Bulg.* **52(2):** 13-24.

Vermeulen, V. C. 1995. Elaborating chironomid deformities as bioindication of toxic sediment stress: the potential application of mixture toxicity concepts. *Ann. Zool. Fenn.* **32:** 265-285.