

# FAILURE IN SOMATIC PAIRING OF 4<sup>TH</sup> CHROMOSOME IN *CHIRONOMUS STRIATIPENNIS* KIEFFER (DIPTERA: CHIRONOMIDAE)

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## KEY WORDS

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## ABSTRACT

Polytene chromosomes of *Chironomus striatipennis* Kieffer were studied in terms of pollution in natural habitats and the polytene chromosomes of the species appeared as paired homologous chromosomes of the flies in the somatic cells at interphase stage. The polytene chromosomes obtained from the flies, collected from polluted environment, exhibited asynapsis along the polytene chromosome arms. Asynapsis along the fourth polytene chromosome in this species was most prominent. The larvae grown in artificially developed polluted condition in the laboratory with addition of Cu as heavy metal in the culture medium also exhibited asynapsis of the fourth polytene chromosome and at higher dose i.e., 15 mg/kg of soil in the substratum of the culture tray the larvae showed almost complete asynapsis of the fourth chromosomes. Hence, the organization of the polytene chromosome may be an index for assessing the polluting condition of the environment where the Chironomid larvae grow.

## INTRODUCTION

*Chironomus striatipennis* Kieffer appears as the most common type of dipterous species found to inhabit almost all ecological habitats (Chaudhuri *et al.*, 1992, Gupta and Kumar, 1991). Their larvae are found to develop in shallow aquatic bodies (Chaudhuri *et al.*, 1992) and at fourth instar stage they develop well organized polytene chromosomes in their salivary gland cells (Gupta and Kumar, 1991). The polytene chromosomes are taken as cytological manifestation of the genetic makeup of the flies and it is well documented fact that the polytene chromosomes respond to stressed condition in the environment (Michailova *et al.*, 2002, 2006, Todorova, 2000). Therefore, the polytene chromosomes obtained from the fourth instar larvae of this species may reveal many intricate phenomena relating to response of the species of *Chironomus* in changed environmental conditions. The present study deals with the impact of heavy metal pollution on the polytene chromosome organization of *C. striatipennis* Kieffer with special reference to its fourth chromosome.

## MATERIALS AND METHODS

Larval samples of Chironomids were collected from their natural habitats containing industrial effluents in urban areas as source of pollution of the aquatic bodies. The study areas included aquatic bodies of Haldia, Kolaghat, Dankuni and Dhapa of West Bengal. The larvae were brought to the laboratory for rearing as well as for preparation of polytene chromosomes from their salivary gland cells. After emergence of the adults the flies were identified for their species status.

The larvae those belong to *C. striatipennis* were taken into consideration for their polytene chromosome study. Soil samples from the sediments of the aquatic bodies (from where the larvae were collected) were also collected for measuring their heavy metal content. At the same time egg masses laid by the gravid adult female flies were allowed to hatch in the laboratory within the artificially developed polluted condition developed by addition of CuSO<sub>4</sub> at different doses as 5 mg, 10 mg, 15 mg and 20 mg per kg of soil in the culture trays. The culture trays were maintained in controlled conditions so that no invasion by predator organisms or other flies could occur in the artificial environment. The fourth instar larvae grown in the culture trays were dissected to obtain their salivary glands and polytene chromosomes were prepared from them. The polytene chromosomes obtained both from the larvae collected from natural habitats as well as from the artificially developed polluted habitats were stained with 0.75% aceto-orcein to get well stained chromosome preparations from the salivary gland cells for microscopic study. At the same time larvae treated with 0.02% colchicine for 2 ½h were dissected for obtaining the brain tissue for metaphase chromosome preparation. After staining with 0.75% aceto-orcein the tissue materials were squashed to get metaphase chromosome plates. The number and organization of the metaphase chromosomes were studied under the microscope.

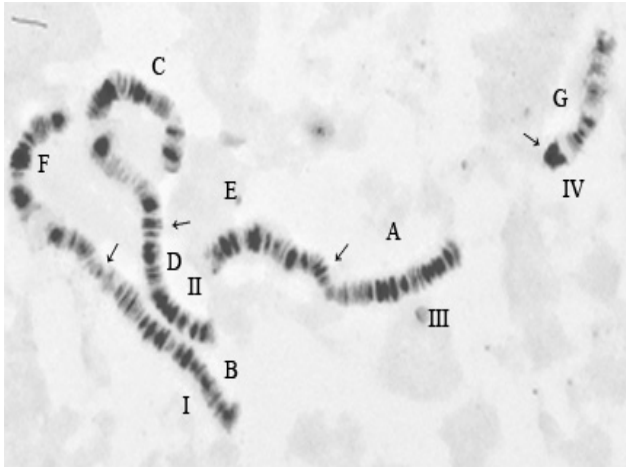
## RESULTS AND DISCUSSION

Each of the salivary gland cells of *Chironomus striatipennis* Kieffer showed the presence of four polytene chromosomes and they were marked as I, II, III and IV according to their

**Table 1: Concentration of heavy metals in the sites of sample collection**

Collection sites	Heavy metal concentration in soil sediments			
	As(mg/L)	Cd(mg/L)	Cu(mg/L)	Pb(mg/L)
Dhapa(Kolkata)	2.04	1.37	103.25	157.36
Kolaghat (TPS )	1.56	<1.0	26.37	109.93
Haldia	3.19	<1.0	32.7	226.43
Dankuni	BDL	0.0018	0.1155	BDL

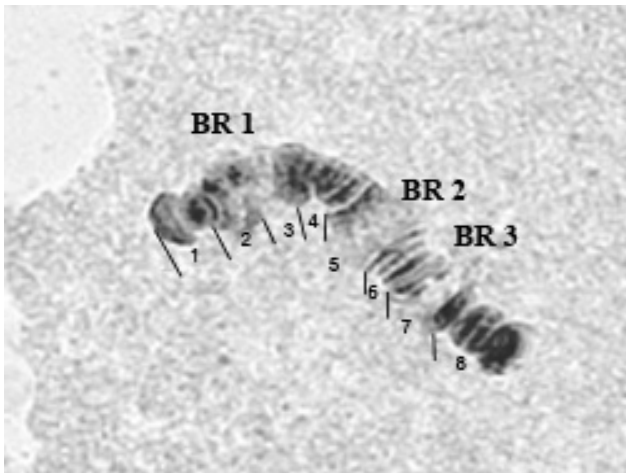
TPS = Thermal Power Station, BDL = Below Detection Limit



**Figure 1: Four polytene chromosomes as observed in *C. striatipennis*. Four chromosomes have been marked as I, II, III and IV**

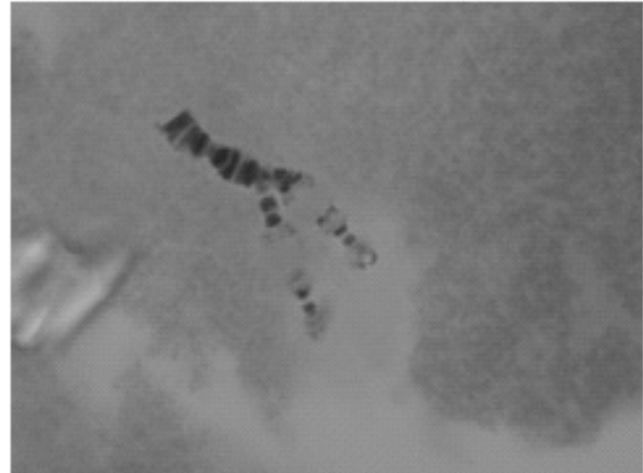


**Figure 2: The metaphase chromosomes of *C. striatipennis* obtained from the neural cell of brain of larva (in the plate there are eight rod like chromosomes)**



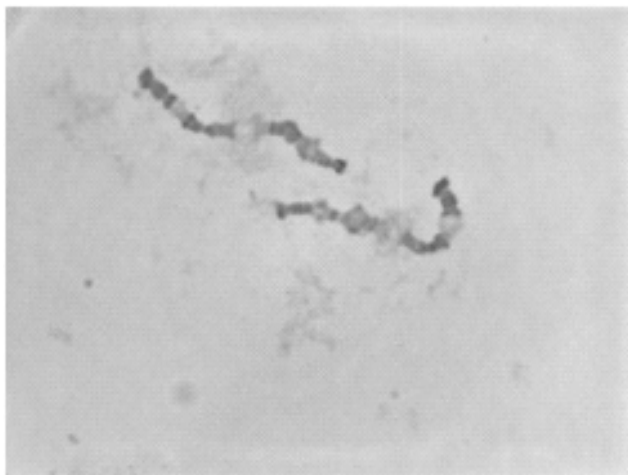
**Figure 3: The fourth polytene chromosome has been mapped. The normal chromosome showed three prominent Balbiani rings marked as BR 1, BR 2 and BR 3. The acrocentric chromosome showed close alignment when the homologues are paired during polytenization.**

decreasing order of length (Fig. 1). This system of designation of the polytene chromosomes was developed by the pioneering investigators on study of polytene chromosomes in chironomids (Devai *et al.*, 1989, Keyl, 1962). On the other hand the neural cells of brain exhibited four pairs of rod like chromosome elements at the metaphase stage (Fig. 2). Hence the diploid chromosome number in this species appeared to be eight and during polytenization the homologous chromosomes underwent pairing in the salivary gland cells of the larvae. Naturally occurring larvae of this species showed



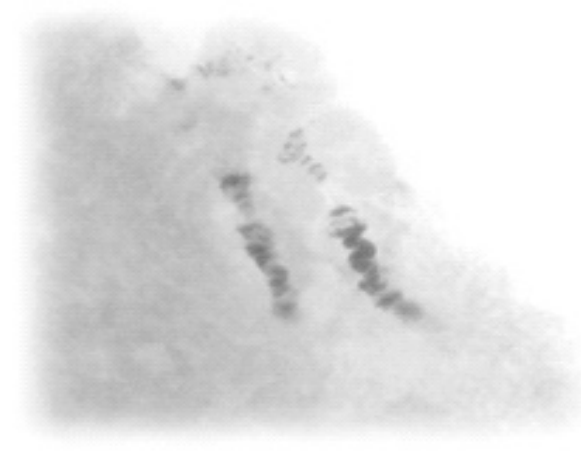
**Figure 4: Partial asynapsis along the acrocentric end of the chromosome**

variable degrees of asynapsis and it became prominent in the fourth chromosome in comparison to any of the other three chromosomes in this species. In some cases the naturally occurring larvae of *C. striatipennis* showed five polytene chromosomes in a salivary gland cell of a larva instead of usual four polytene chromosomes in a cell and the additional chromosome appeared to be the fourth chromosome (Fig. 5). Therefore, the additional fourth chromosome in this case marked as failure in somatic pairing of the fourth chromosomes during their polytene chromosome formation. The pollution level of the selected zones was also measured with relation to their heavy metal concentration and an estimate of this study may be presented through the following Table 1. As the



**Figure 5: Two fourth chromosomes in polytene form failed in somatic pairing during polytenization**

Chironomid larvae live in water inside tubes made of their silk secreted from the salivary glands and mud at the substratum as well as the larvae feed on the organic decomposed materials in the sediments, an estimate of the heavy metal concentration of the soil sediment was taken into consideration in the present study. Comparison of the heavy metal contents in the habitats under consideration and the reference limit of the respective heavy metals may be judged to realize the level of pollution in the selected aquatic zones. The guideline values for the heavy metals namely As, Cd, Cu and Pb are known to be 0.01, 0.003, 2 and 0.01 mg/L respectively. Hence, the selected zones of study in this case appeared to be highly polluted. However, among several heavy metals studied for their presence in the soil sediments, the concentration of Cu appeared to be quite high (Table 1). An attempt was made to correlate the high concentration of Cu in the habitats with organization of the fourth polytene chromosomes in *C. striatipennis*. The preparations from the larvae grown in the laboratory under artificially developed polluted condition showed a remarkable feature with regard to the organization of the fourth chromosome in the species, when at higher doses of Cu concentration (15 mg/kg of soil and above), the fourth chromosomes failed to pair and in separated condition they underwent polytenization (Fig. 6). Hence, the high concentration of copper in the medium appears to be related to non-pairing of the fourth chromosomes during polytene chromosome formation. To analyze the organization of the fourth chromosome it may be pointed out that this chromosome appeared acrocentric with three Balbiani rings at different positions (Fig. 3). The nucleolar organizer was found to be located at the acrocentric terminal of the chromosome (Gupta and Kumar, 1991). Pairing of the homologous fourth chromosome during the polytene chromosome formation like all the other chromosomes probably gives an additional support to the chromosome to show vigorous gene activity in a conjoint fashion during embryonic development promoting rapid embryonic differentiation in this species. In this consideration asynapsis along the chromosome arms appears to be an abnormal and unusual condition (Fig. 4). The fourth chromosome was the shortest among the four chromosome complements and it



**Figure 6: Failure of two fourth polytene chromosomes in a plate from somatic pairing at higher dose of Cu induction**

appeared to be acrocentric (Sarkar *et al.*, 2011, Gupta and Kumar, (1991). Asynapsis and other aberrations along the chromosomes are considered as an influence of pollution in the environment by many investigators (Aziz *et al.*, 1991, Michailova, 2002, Michailova *et al.*, 2002, 2006, Tachi and Nishime, 1975, Todorova, 2000). Compared to the normal configuration of the fourth polytene chromosome, the unusual detached fourth chromosomes showed more heterochromatic zones with less puffing activities. In this regard it may be surmised that high level of copper concentration in the culture medium somehow affected the expression of some prominent genic regions that led to asynapsis of the smallest acrocentric fourth chromosome. However, such an impact of pollution appeared to be maximum on the fourth chromosome as they failed completely to undergo synapsis. The other chromosomes though showed asynapsis but such asynapsis never affected the whole chromosome. In this consideration the fourth chromosome may be taken as the best tool for monitoring the aquatic pollution.

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