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A STUDY ON THE POLYMORPHIC FOURTH CHROMOSOME OF *CHIRONOMUS STRIATIPENNIS* (KIEFFER)

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ABSTRACT
Chironomus striatipennis(Kieffer) contains four well organized polytene chromosomes in a salivary gland cell of the fourth instar larva. Out of the four chromosomes the fourth one is acrocentric and smallest in size.The fourth chromosome in this species showed various polymorphic organizations in the specimens collected from different habitats with differential heavy metal concentrations suggesting a differential response of this chromosome under different conditions. The polymorphic organizations also indicated the presence of some weak zones at several regions of the chromosome those may promote differential organization of the chromosome in this species.

INTRODUCTION

Chironomids represent one type of Dipteran fly and they develop polytene chromosomes in many of their somatic cells during their larval life. Because of the possession of polytene chromosomes the chironomid larvae have achieved enough importance for studies in Cytogenetic and Molecular Biology (Martin, 1969; Michailova, 1989; Petrova *et al.,* 2007). In most of the species of *Chironomus* there are four polytenized chromosomes in the salivary gland cell of the fourth instar larva (Keyl, 1962; Michailova,1989; Kiknadze *et al*., 1991). Among the four chromosomes the fourth chromosome is the smallest one and it is acrocentric. An attempt was made to study the polymorphism of the fourth chromosome of *Chironomus stratipennis* Kieffer to reveal their mode of origin. This on the other hand suggested the organization of this chromosome in the species.

MATERIALS AND METHODS

The larvae of the species *Chironomus striatipennis* (Kieffer) collected from different aquatic habitats of Dhapa of Kolkata area, Haldia and Kolaghat on East Midnapore, Deulti and Dhulagarh of Howrah and Durgapur of Burdwan district. All the habitats belong to either industrial zones or having high pollution level because of dumping of waste materials. The larvae were brought to the laboratory and fourth instar larvae were taken from the mass for chromosome preparation. From the collection sites water and soil samples were also collected for estimating their heavy metal contents by Atomic Absorption Spectrophotometry in order to measure the level of pollution of the habitats. From the fourth instar larvae the salivary glands were dissected out and they were fixed with aceto-methanol before preparation of the polytene chromosomes from the cells. The gland materials are then stained with 2% aceto-orcein.The stained gland tissues were squashed on the clean glass slides following standard methods. Besides some slides were also prepared having squash of the unstained gland tissues for C- banding and permanent preparations.The squash preparations were then observed under high power objectives to reveal the organization of the polytene chromosomes. Special emphasis was given to the study on the organization of the fourth chromosome in the cell. Mapping of the fourth chromosome was done following the method of Kerkis *et al*., 1989.

RESULTS

Chironomus striatipennis (Kieffer) showed the presence of four well organized polytene chromosomes with distinct display of bands and interbands. The fourth chromosome recognized as the G arm appeared smallest in which the normal configuration of the chromosome showed eight prominent segments (Fig. 1a). At the segment 3, 5 and 7 there were three distinct Balbiani rings. Several polymorphic forms of the fourth chromosome were obtained in the samples collected from natural habitats and they were designated as Ab 1 (Fig.1b), Ab 2 (Fig.1c), Ab 3 (Fig.1d), Ab 4 (Fig.1e), Ab 5 (Fig.1f.), Ab 6 (Fig.1g), Ab 7(Fig. 1h), Ab8(Fig.1i) and Ab 9 (Fig.1j). The normal texture of the 4th polytene chromosome and the different variant

forms as observed in *C. striatipennis* may be characterized in the following way (Table 1).

These aberrant forms could be grouped into three main categories as apparently normal with minor inversion (Fig.1b), the aberrant looped form with heterozygous inversion (Fig.1c) and asynaptic form(Fig.1d to 1j). The asynaptic forms appeared to be maximum of the polymorphic forms found to be present in all the samples collected from different habitats. It has also been mentioned that the sites from where the specimens were collected were found to be having high level of heavy metal content. An estimate of the heavy metal content of the collection sites both in the soil and water samples of the aquatic habitats of the larvae may be given in the following Table 2.

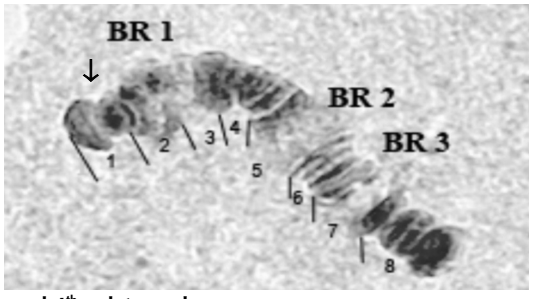
DISCUSSION

Conventionally the polytene chromosomes appear in the interphase nucleus of *Chironomus* in their larval salivary gland cells followed by somatic pairing(Beermann, 1971). Hence, there are four polytene chromosomes in a cell in stead of eight chromosomes as in diploid set. The fourth chromosome of the set is acrocentric and shortest by dimension having clear and landmark divisions(Kerkis *et al*., 1989; Michailova *et al*., 1996, 1998, 2001, 2003). But this chromosome was found to exhibit significant morphological variations in *C. striatipennis*, when the other chromosomes were not so sensitive to produce polymorphic configurations. This suggests a greater sensitivity of the fourth chromosome to external stress than any of the other three chromosomes. This, on the other hand, suggests its simpler organization in consideration to its chromatin content. The shortest acrocentric chromosome with its eight small divisions and three Balbiani ring components appear to be the simplest configuration maintained for long time in course of biological evolution. When due to somatic pairing two homologous fourth chromosomes come closer forming long banded filamentous structure, the presence of three Balbiani ring segments at the adjacent locations probably make them less adhesive than the other counterparts of the set. The paired existence of the homologous chromosomes in this case though exerts a conjoint effort to show expression of several of its genes together, but the presence of pollutants in the environment probably has been a hindrance to their cooperativity. The impact of heavy metals resulting chromosomal polymorphism in Chironomids was also observed by other investigators (Aziz *et al*.,1991; Michailova, 2002; Michailova *et. al*., 2002, 2003; Sharma *et al*.,1988; Tachi and Nishime, 1975; Todorova, 2000; Wistel *et al*., 1978). In this concern the polymorphic condition of the fourth chromosome only may be an indication of pollution level in the aquatic environment.

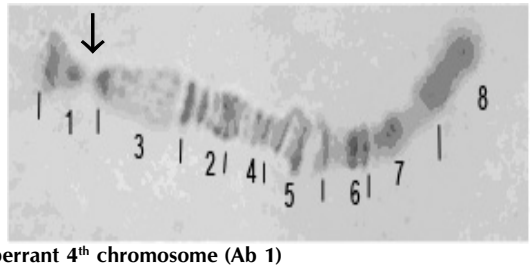
Considering all the morphological variations of the fourth chromosome in its polytenized configuration in this species it may be concluded that the variable forms of chromosome belong to three classes namely homozygous inversion, heterozygous inversion and asynapsis. In the present investigation Ab 1 belongs to homozygous inversion in which segment 2–3 in the polytene chromosome was found to be inverted, the second type is represented by Ab 2 with inversion along one of the homologues of the fourth chromosome

Table 1: The Normal and aberrant fourth chromosomes of *C. striatipennis*

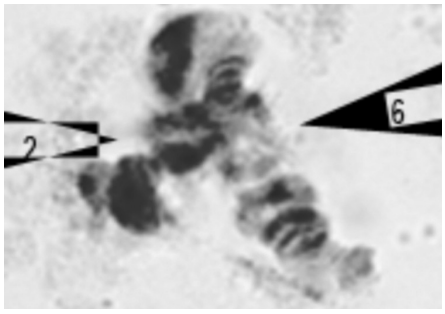
Fourth chromsosome of *C. striatipennis*	Salient features	Significant points
Normal form	Acrocentric in structure with8 prominent segments,Balbiani rings at segment 3, 5 and 7,Segment 7 with nucleolar organizer (Fig.1a).	Highly sensitive to environmental condition.
Aberrant Form 1 (Ab 1)	Normal in appearance but with a short homozygous inversion involving segments 2-3.(Fig. 1 b)	The structure probably appeared under stressed condition.
Aberrant Form 2 (Ab 2)	Severely aberrant in appearance with looped configuration due to heterozygous inversion at the middle involving segment 2-6.(Fig.1 c)	Genetic heterogeneity to tolerate the unfavourable condition due to pollution.
Aberrant Form 3 (Ab 3)	Terminal asynapsis along segment 7 and 8, Nucleolar organizer region appeared at the segment 7 as two highly swelled region.(Fig. 1 d)	Short deletions at one of the chromosome of the two homologues of the 4th chromosome made them separated without pairing.
Aberrant Form 4 (Ab 4)	Moderately long asynaptic Form with major asynapsis, Balbiani rings and nucleolar organizers absent (Fig. 1e).	Unequal heterochromatinization and loss of segments appeared to be the major feature for asynapsis.
Aberrant Form 5 (Ab 5)	Major part of the chromosome showed synapsis, only a small segment at the terminal side located far from the centromere showed asynapsis (Fig.1f).	A small deletion at the terminal segment of one of the two homologues probably resulted such configuration.
Aberrant Form 6 (Ab 6)	The chromosome showed moderate intercalary asynapsis and short adcentromeric asynapsis (Fig. 1g)	Balbiani rings at segment 7 appeared prominent and segment 8 with diffused chromatin.
Aberrant Form 7 (Ab 7)	The chromosome with long asynaptic arms and synapsis retained only at segment 1 (Fig.1h).	Unequal puffing activity noticed at the segment 3, other regions appeared mostly heterochromatinized.
Aberrant Form 8 (Ab8)	The chromosome showed long asynapsis involving segments from 2 – 8, synapsis onserved only along segment 1 and part of segment 2 (Fig.1i)	Unequal puffing activity noticed along segment 7 and 8.
Aberrant Form 9 (Ab 9)	The chromosome with asynapsis at both sides of the centromere. At one side asynapsis extended from segment 3 to 8 and at the other side asynapsis remained restricted to segment 1(Fig. 1 j)	Puffing activity observed at all the three regions as observed in the normal G arm of the chromosome.



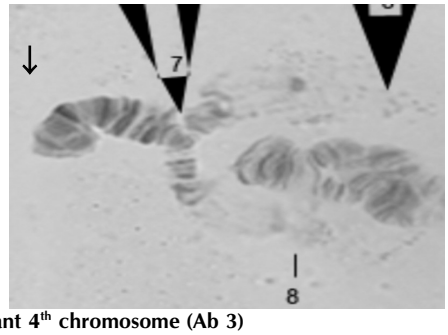
(a) Normal 4th polytene chromosome



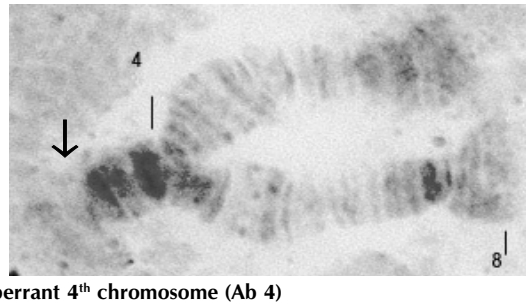
(b) Aberrant 4th chromosome (Ab 1)



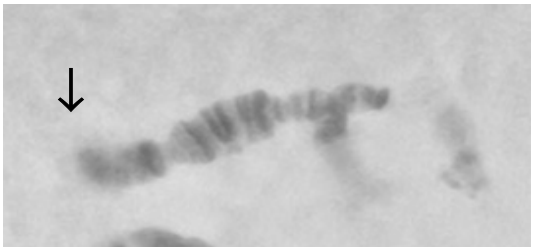
(c) Aberrant 4th chromosome (Ab 2)



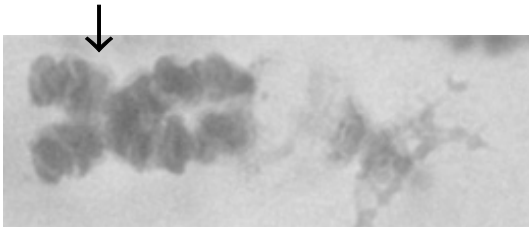
(d) Aberrant 4th chromosome (Ab 3)



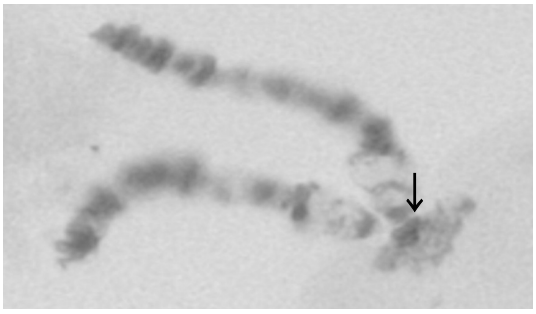
(e) Aberrant 4th chromosome (Ab 4)



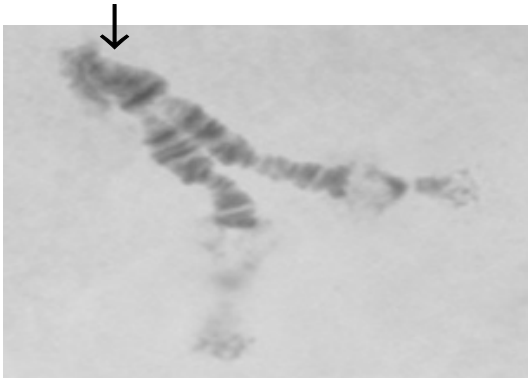
(f) Aberrant 4th chromosome short asynapsis and terminal deletion (Ab 5)



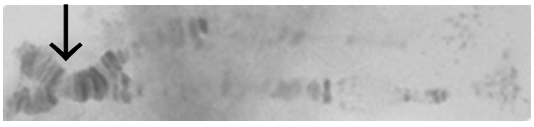
(g) Intercalary asynapsis (Ab 6)



(h) Aberrant 4th chromosome: long asynapsis (Ab 7)



(i) Aberrant 4th chromosome long asynapsis showing subterminal gene activity (Ab 8)



(j) Aberrant 4th chromosome with both short and long asynapsis (Ab 9)

Figure 1: Normal and polymorphic fourth polytene chromosome of *Chironomus striatipennis*. There are 9 aberrant forms of fourth polytene chromosome of the midge marked as Ab 1 to Ab 9. The arrow in the chromosome figure indicates the centromeric position. The different segments of the chromosome are marked by Arabic numerals.

Table 2: Heavy metal contents of the aquatic bodies in the collection sites

Collection sites	Heavy metal concentration									
	As(mg/L)		Cd(mg/L)		Cu(mg/L)		Pb(mg/L)		Hg(mg/L)	
	Wat.	Sed.	Wat.	Sed.	Wat.	Sed.	Wat.	Sed.	Wat.	Sed.
Dhapa(Kolkata)	<.005	2.04	<.005	1.37	.03	103.25	.008	157.36	<.001	.64
Kolaghat (TPS)	<.005	1.56	<.005	<1.0	<.01	26.37	.008	109.93		<.5
Haldia	<.005	3.19	<.005	<1.0	<.01	32.7	008	226.43	<.001	<.5
Deulti	.006	3.42	<.005	<1.0	<.01	23.53	<.005	15.47	<.001	<.5
Damodar:Durgapur	3.3121	7.7383	BDL	BDL	BDL	0.0278	BDL	BDL	0.0705	0.0047
Dhulagar	7.0229	19.7388	BDL	0.0020	0.0020	0.0561	BDL	BDL	0.0166	0.0188

Wat. = Water; Sed. = Sediment, BDL = Below detectable limit, TPS = Thermal Power Station

involving the segments 2 – 6, because of which the chromosome attained a looped configuration (Fig. 1c). However, there were several varieties in the asynaptic category which include Ab 3, Ab 4, Ab 5, Ab 6, Ab 7, Ab 8 and Ab 9 (Fig. 1d – 1j). These figures show gradual increase in the degree of asynapsis starting from short terminal segment to the extreme involving almost whole of the G arm (the 4th chromosome) except the terminal centromeric region. In certain cases asynapsis was found to be inserted at both the ends of the chromosome, besides the intercalary asynapsis (Fig. 1 g and 1j).

The different polymorphic forms of the fourth polytene chromosome in this species suggest its response to the environmental conditions (Sharma *et al*., 1988; Aziz *et al*., 1991; Michailova, 2002; Michailova *et al*., 2002, 2003). All the habitats taken under consideration were polluted with different heavy metals such as As, Cd, Cu, Pb and Hg and therefore, the aquatic bodies as habitats of the species of *Chironomus* became incompatible for inhabitation. Under the pressure from toxic metal pollutants chromosomal normal functioning was hampered and this was expressed through variable organization of the same chromosome of the species inhabiting the polluted aquatic bodies. One cytological expression of the activity of the normal fourth chromosome in its polytene form is the formation of distinct Balbiani rings along the chromosome arm. In most of the variable forms where the phenomenon of asynapsis resulted variable morphologies, either the puffing activity has been greatly affected without the formation of a Balbiani ring or reduction in puffing activity indicating loss of one or two Balbiani rings from the chromosome arm.

From all these findings several questions are emanated such as why the 4th polytene chromosome appear more prone to polymorphic organization, what makes the polytene bivalents to remain in paired condition and why sometimes the polytene figures show partial asyanapsis and whether asynapsis may lead to heterologous gene activity along the separated chromosome homologues. The polymorphic configurations of the fourth polytene chromosomes of *C. striatipennis* throw some light on the organization of the polytene chromosomes and give some plausible answers of the questions already mentioned here. The asynaptic polytene segments of the chromosome states that the chromatin threads are developed on a homogeneous platform extending along the whole length of the chromosome arm and the environmental stress may separate this platform into two strings on which the separate homologous threads, the precursors for the polytene chromosome, may continue replication causing asynapsis along certain sections of the chromosome arms. Further in the polytene chromosome formation the centromeric cohesion appears stronger than the cohesion along other regions of the chromosome. The chromatin content of the fourth polytene chromosome of *Chironomus* appeared distinctly different from the other chromosomes and therefore, this chromosome appeared more responsive to environmental stress. In fact the more the mass of heterochromatin in a chromosome the more will be its tolerance to stress. In this consideration the fourth chromosome of *Chironomus* being shortest one and acrocentric having less amount of heterochromatin content becomes more sensitive to environmental stress. Differential puffing activities along the asynaptic region of the chromosome appears to be supportive to heterogenous gene activities of the chromosome homologues which probably give better adaptability to the species in stressed condition of the environment.

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