Int. J. Exp. Res. Rev., Vol. 3: 14-29 (2016)

International Journal of Experimental Research and Review (IJERR)
©Copyright by International Academic Publishing House (IAPH)
ISSN: 2455-4855 (Online) ***Review Article***
Received: 8th January, 2016; **Accepted:** 4th February, 2016; **Published:** 28th February, 2016

DOI: https://doi.org/10.52756/ijerr.2016.v03.003

Effects of heavy metal pollutants on polytene chromosomes of Chironomid larvae

Chinmoy Ghosh

Department of Zoology, Basirhat College, Basirhat, North 24 parganas, West Bengal, India

Author's E-mail: chinmoyghosh1981@gmail.com

Abstract

The non-biting midges of genus *Chironomus* are well known biological indicator and widely used test organism for water quality studies. The larvae of this most abundant and widely distributed species of aquatic ecosystem are characterized by the presence of giant polytene chromosomes with very good banding pattern. The karyological characteristics of polytene chromosome have been used to show chromosomal aberration under action of heavy metal pollutants like copper, cadmium, lead, chromium, etc. Both structural and functional changes in response to heavy-metals have been shown in polytene chromosomes of chironomid larvae of different species from anthropogenically polluted water bodies and in laboratory cultures. Structural changes in response to heavy metal involve numerous inversions, deletions, duplications, deficiencies, centromeric breakages, telomere condensations/ de-condensations, amplification of some polytene areas, desynapses, alteration in puffing pattern etc. In some chironomid species it has been shown that the fourth chromosome has transformed into so called pompon due to heavy metal toxicity. Functional changes due to effect of heavy metal include higher or lower activity of puffs and Balbiani ring (BR) system and changes in activity of Nuclear Organizer Region (NOR). All these responses have shown to be species specific and dose dependent. It has been also shown that the sites of chromosomal rearrangements are associated with certain chromosomal breakpoints which are rich in repetitive DNA sequences. All these structural and functional changes of polytene chromosome system could be used as a tool for assessment of genotoxicity in aquatic ecosystems which can provide an early warning of adverse long term effects of heavy metals on aquatic organisms.

Keywords: Chironomids, biomarker, heavy metal, polytene chromosome.

Introduction

Environment is defined as the totality of circumstances surrounding an organism or group of organisms' specially, the combination of external physical conditions that affect and influence the growth, development and survival of organisms (Farlex, 2005). It consists of the flora, fauna and the abiotic factors, and includes the aquatic, terrestrial and atmospheric habitats. A pollutant is any substance in the environment, which causes objectionable effects, impairing the welfare of the environment, reducing the quality of life and may eventually cause death. Such a substance has to be present in the environment beyond a set or tolerance limit, which could be either a desirable or acceptable limit. Hence, environmental pollution is the presence of a pollutant in the environment which may be poisonous or toxic and will cause harm to living things in the polluted environment. The term “heavy metals” refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration (Lenntech, 2004). “Heavy metals” is a general collective term, which applies to the group of metals and metalloids with atomic density greater than 4 g/cm^3, or 5 times or more, greater than water (Huton and Symon, 1986). However, being a heavy metal has little to do with density but concerns chemical properties. Heavy metals include lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag) chromium (Cr), copper (Cu) iron (Fe), and the platinum group elements. Some elements like iron, zinc, copper, cobalt, chromium, nickel are needed in small quantities for metabolism, but may be toxic at higher levels. Others like lead, mercury, cadmium, and arsenic etc. have no beneficial role and are positively toxic. Effects of heavy metals can be deleterious when they are released in water either in high concentrations for short periods, causing acute toxicity, or in low concentrations but for long periods, causing chronic toxicity and leading to disorders in development, growth, maturation, reproduction, hatching, which not necessarily result in early death.

Environmental pollution from such hazardous metals and minerals can arise from natural as well as anthropogenic sources. Natural sources are: seepage from rocks into water, volcanic activity, forest fires etc. Pollution also arises from partitioning of polluting elements (which are concentrated in clay minerals with high absorption capacities), between sedimentary rocks and their precursor sediments and water. With rapid industrialization and consumerist life style, anthropogenic sources of environmental pollution have increased. The pollution occurs both at the level of industrial production as well as end use of the products and run-off. These toxic elements enter the human body mostly through food and water and to a lesser extent through inhalation of polluted air, use of cosmetics, drugs, poor quality herbal formulations particularly herbo-mineral preparations, and even items like toys which have paints containing lead.

Heavy metal pollution: from global to local

The problem of heavy metal pollution is a matter of serious concern on a local, regional and global scale. There was a certain rate of emission of heavy metals in to environment by natural means like volcanic activity and erosion. But the rate has been dramatically accentuated since the beginning of industrial revolution in the developed countries and the scenario is becoming worse day by day with the rapid pace of industrialization, urbanization, mining activity and use of synthetic pesticides and fertilizers in developing and underdeveloped countries. US Environmental Protection Agency (EPA) and US Agency for Toxic Substances and Disease Registry (ATSDR) has been listed many metals as priority pollutants: where Lead, Mercury, Arsenic and Cadmium are categorized as first, second, third and sixth hazardous metals. In Japan more than thousand people have died due to Minamata disease caused by mercury pollution. Italy and Bangladesh is facing severe problem from Arsenic contamination of Ground water (Alam et al., 2003). The countries like Bolivia, Hong Kong and Germany are experiencing problem of drinking water pollution by heavy metals like Cadmium, Lead, Copper and Zinc (Miller et al., 2004). Lead, Copper, Chromium and Cadmium toxicity has also been reported from Turkey (Onder et al., 2007). In Senegal acute Lead poisoning due to lead dust and soil exposure from battery recycling leads to child death in an unexpected catastrophe (Haefliger et al., 2009). China is also facing soil contamination problem from heavy metal pollution (Brus et al., 2009). As far as India is concerned, the picture of heavy metal contamination is equally gloomy. The major hazardous metals of concern for India in terms of their environmental load and health effects are lead, mercury, chromium, arsenic and copper. Press Information Bureau, Govt. of India has a page in their website displaying the Major Heavy Metal Contaminated Sites in India which shows that Gujarat, Maharashtra and Andhra Pradesh contribute to 80% of hazardous waste (including heavy metals) in India. The Chromium contaminated places of India includes Ranipet, Tamil Nadu; Kanpur, Uttar Pradesh; Vadodara, Gujarat; and Talcher Orissa. Ratlam, Madhya Pradesh; Bandalamottu Mines, Andhra Pradesh; Vadodara, Gujarat, Korba, Chattisgarh are contaminated with Lead. Mercury and copper contamination have been found in Madhya Pradesh, Tamil Nadu, Jharkhand and Orissa. Pockets in West Bengal, Bihar, UP, Assam and Chhattisgarh are the major states affected by arsenic contamination of water, West Bengal being by far the worst affected. Ground water of 9 out of 16 districts of West Bengal is heavily contaminated with arsenic, affecting 26 million people (Chakroborty et al., 2009). Presently a vast alluvial track of Bengal delta plain is seriously affected by the arsenic contamination in ground water. Annual Report of West Bengal Pollution Control Board, 2008 also shows that Iron, Mercury, Zinc and Lead concentration of some stations of West Bengal is beyond permissible limit. A study on brackish water from the Bidyadhari River of north Sundarbans shows cadmium concentration is alarmingly higher (Saha et al., 2001). Another important concern is the pollution of River Ganga, which is considered lifeline of India. Recent reports shows that lead, cadmium and nickel concentration at various places of Ganga, are higher than their respective maximum admissible concentration (Pandey et al., 2009).

Monitoring of heavy metal pollution: use of bioindicators

Regarding the growing problem of heavy metal pollution it is becoming necessary to monitor the concentration of heavy metal in environment in regular basis. Monitoring and systemic gathering of information are essential component of any pollution control system. The level of the pollution can be determined by different approaches like physical, chemical and bio-indication methods. In recent years there has been growing interest in using bio-indicators for monitoring environmental pollution with heavy and toxic metals. Bioindicators are organisms (animals, plants or bacteria) used to monitor pollutants of an environment or ecosystem. They are any biological species or group of species whose function, population, or status can be used to determine ecosystem or environmental integrity. The principle behind the bioindicator approach is the analysis of an organism heavy metal contents and compared the metal concentration with the background metal levels. Burger (2006) has shown in a review concerned with the used of bioindicators that, over 40% of the bioindicator papers were about metal pollution and in those paper the most used bioindicators are plants, invertebrates, fish and mammals. For aquatic metal pollution, the common used bioindicators mainly contained organisms including crustaceans, insects, mollusks, fish, plant, bird etc. Among the crustacean Daphniidae toxicity test is an essential assay for worldwide water quality assessment based on the strong phototactic behavior in *Daphnia magna* (Meester, 1993). Shrimps are also used to assess the bio accumulation pattern of heavy metals. Mitra et al., (2010) have shown in gangetic shrimp near Haldia petrochemicals, West Bengal that they have become unsuitable for human consumption due to heavy metal accumulation. Temara et al., (1998) in Norway used common starfishes as bioindicator of cadmium, lead and zinc pollution. Bivalve mollusks, as filter-feeding organisms, are known to accumulate metals that can produce deleterious effects on organisms. So the oysters, mussels and clams are used as the bio-monitors for the evaluation of heavy metals pollution in marine waters (Beldi et al., 2006). Fishes have also attracted much attention in biomonitoring of heavy metals; as for example Chinese loach has been used for genotoxicity test of cadmium, lead and zinc (Yingmei et al., 2008). Among the insects the chironomid flies are EPA approved test organism for sediment toxicity test and regularly used as bioindicator of heavy metal pollution. These review concerned with change in polytene chromosome of chiromid flies in response to several heavy metals.

Chironomids: a good model organism to test genotoxicity of heavy metals

Chironomids (Diptera: Chironomidae) are an especially important component of benthic aquatic communities. They are one of the most dominant, widespread, and diverse aquatic invertebrate taxa in freshwater systems (Armittage, 1995). Chironomids are an important food source to larger predatory invertebrates, fishes, and birds and are important contributors of carbon and energy flow to higher trophic levels (Benke and Wallace, 1997). Chironomids are holometabolous insects. Their life cycle includes three aquatic developmental stages (egg, larva, and pupa) and a terrestrial reproductive stage (winged adult). Although the duration and attributes of each life stage are species-specific, chironomids spend most of their lives as benthic larvae living and feeding in or on the sediment [or macrophytes] (Oliver, 1971). Because of their close association with the benthic zone, easily identifiable life stages, ease of culture, and sensitivity to chemical and environmental stressors, chironomid larvae are commonly used as indicator species in laboratory and field-based toxicity tests (Environment Canada, 1997). They exhibit both developmental (Timmermans et al., 1992; Dube and Culp 1996) and teratogenic (Martinez et al., 2001) responses to a wide range of chemical contaminants. Chironomid larvae also reflect chemical stress by exhibiting structural (Michailova et al., 1998) and functional changes in the polytene chromosomes of their salivary glands (Aziz et al., 1991; Michailova et al., 1998; Planello et al., 2007).

Most of the studies for genotoxicity of the chironomids have been done in Europe and Australia and recently there are a few studies from India. The species involved in such studies includes the genus *Chironomus* and *Glyptotendipes*. It is important that the species can be reproduced in the laboratory

(Michailova, 1985) and used in dose-response experiments in order to validate and calibrate responses observed in the field. Such species are *Chironomus riparius*, *C. piger*, *Glyptotendipes barbipes*, *G. salinus*, *G. glaucus* and *G. pallens* (Ilkova, 2004). The response of the genome to various stress agents was also detected in *C. bernensis* (Petrova, Michailova, 2002; Michailova et al., 2009b) and *C. plumosu* (Michailova, Mettinen, 2000; Ilkova, 2004; Michailova et al., 2009b). Also, the reaction of *C. acidophilus* genome from an acidic lake was analyzed (Michailova et al., 2009a). In India several studies on the effect of heavy metals on polytene chromosomes have been done using *Chironomus striatipennis* Kieffer as model organism (Bhaduri et al., 2011, Sarkar et al., 2011). In Australia such studies has been done on *Chironomus duplex* and *Chironomus dilutes* (Martin et al., 2010). Knowledge of the biology of the species, their phylogeny, and DNA organization of their genome is very important in consideration of genomic responses. The polytene chromosomes of the above species have clear band structures, and good standard chromosome maps are available for them (Hägele, 1970; Kiknadze et al., 1991). This allows tracing the cytogenetic effects of some metals on the structure and function of polytene chromosomes. Cytogenetic effects can also be studied under laboratory conditions, and typical chromosome rearrangements are known to be signals for certain pollutants.

Structure of Polytene chromosome of *Chironomus*

Polytene chromosomes are giant interphase chromosomes found in larval salivary glands of dipterans insects. In properly stained preparation they show characteristic bands and inter-bands along the lengths of the chromosomes. In Chironomidae the arrangement of these bands with some other chromosomal characteristics shows species specific variation and could be used for identification of species. A typical haploid karyotype of the species belonging to the genus *Chironomus* comprises four chromosomes formed by seven chromosomal arms A, B, C, D, E, F and G. Reciprocal translocation of these whole arms have important role in evolution of different species of genus *Chironomus*. Some other process of chromosomal rearrangement like paracentric inversion, fusion of chromosomal arms, local amplification of centromeric DNA, and change in number and location of nucleolar organizer have played important role in the evolution of new chromosomal combination in different species. Currently there are two types of system for banding of *Chironomus* chromosome: some authors works with Keyl-Devai system (Devai et al., 1989) whereas others prefer Maximova or Maximova-shobanov system (Maximova, 1976). H. G Keyl was the first who prepared standard cytogenetical map of arm A, E and F of polytene chromosome of *Chironomus piger,* Strenzke, a959 and mapped banding sequences of some other species of genus *Chironomuus* (Keyl 1962). Later Devai with coauthors (Devai et al., 1989) developed standard maps of for arm B, C and D. Keyl – Devai mapping system is used for the mapping of all species from the genus *Chironomus* with banding sequences of *C. piger* as a standard. In this mapping system chromosomal arm is divided into regions, which are designated by numbers. Inside every region bands are designated by letters. Thus every band of chromosomal arm has exact designation. However, there is significant inter-specific divergence of banding sequences in arm B and G in other

Chironomus species studied. So it is impossible to make complete maps of these two arms of all *Chironomus* species in Keyl-Devai system. In Maximova system (Maximova 1976) banding pattern of *Ch. plumosus* is standard for mapping of other species from *C. plumosus* group. Although standard maps were created for all 7 chromosomal arms, the Maximova system has grave disadvantage, as there is no exact designation for single bands as it is in Keyl-Devai mapping system. As a result in all cases when inversion breakpoints fall inside a region instead of its border, the resulted of this region are designated arbitrarily. In 1994 N. A Shobanov made a revision of Maximova mapping system and gave exact designation to all the bands. Nevertheless, converting one mapping system to other is difficult because of the number of designated bands in the two systems. This number difference is due to the different label of chromosome compactization and resultant number of visible bands. So it is impossible to compare banding sequences of *C. plumosus* sibling species without comparison of chromosome photos. Keyl (1962) has divided the genus *Chironomus* into five groups recognizable by five different whole arm combinations.

CD BF E.

AB	CD	EF	Thummi
AB	CF	DE	Campto- *Chironomus*
AC	BF	DE	Para-thummi
AD	BC	EF	Commutatus / Lacunarius
AE	BF	CD	Pseudo-thummi
AF	BE	CD	Matures

Among all possible combination of six arms only five was found by Keyl as is evident from the table. Later on another arm combination was noted by Wuelker et al., (1968). In these entire cases G arm remain separate as fourth chromosome. Kiknadze (2008) has mentioned another arm combination Columbiensis: AG

Changes in *Chironomus* polytene chromosome due to heavy metal pollutants

For studying the changes of polytene chromosome due to heavy metal pollutants generally two approaches are taken. In one approach chironomid larvae are collected from heavy metal polluted water body and polytene chromosome from such larvae are studied. The revealed aberrant changes in respect to normal karyotype are correlated with the heavy metal concentrations of water or sediment of the same water body. In another approach normal larvae are cultured in the laboratory environment and such stocks are exposed to the varying levels of specific heavy metal under controlled condition of laboratory culture. Comparison of control larvae with the experimental one reveals the changes of chromosome in response to specific heavy metal. The studies of aberration of chironomid chromosomes revealed that there are two types of chromosomal changes can take place. Inherited aberrations: Here the chromosomes are equally affected in all cells of both salivary glands and they are inherited to the next generation. Somatic rearrangements: Here only few cell of salivary gland of one individual is affected and the aberrations do not inherited to the next generation. Again from another aspect the changes may be of two types. Structural changes: Here the overall structure or banding pattern of chromosomes is changed due to action of heavy metals. It generally involves chromosome breakage and reunion or chromatid separation. Another type is Functional changes where gross chromosomal structure remains intact but changes take place in functional level e.g., appearance of new puff due to change in gene expression.

Structural Changes

Inversion

An inversion is a chromosome rearrangement in which a segment of a chromosome is reversed end to end. An inversion occurs when a single chromosome undergoes breakage and rearrangement within itself. Inversions are of two types: paracentric and pericentric.

Inherited inversions

Most of the inherited inversions found in the European populations of chironomids collected from heavy metal polluted regions are of heterozygous paracentric type. Except *C. riparius* the frequency of this type of inversion is more than 1% (Petrova and Michailova, 2002, Michailova et al., 2009a, b). In *C. riparius* the inversion frequency is less than 1% (Kiknadze et al., 1988; Sella et al., 2004). Most of the cases the inversion took large segment of one arm of the chromosome. The observed heterozygous frequency in these European populations does not differ significantly from that expected from Hardy-Weinberg Equilibrium. Again no correlation was established between concentrations of heavy metals and observed inversion frequency (Sella et al., 2004).

All the inherited inversions were in heterozygous state and paracentric apart from a few pericentric inversions in chromosome EF. Inversion variants were found in all chromosome arms. Arm F was the most strongly polymorphic with 16 different types of inversions. It was followed by arms B and C with 15, arm E with 10, arm A with four and arm G with one inversion only. Most of the inversions were present in only one out of the 13 populations. Only six inherited heterozygous inversions were shared by two populations (Sella et al., 2004). All the observed inherited inversions occurred in very low frequency. The median percent frequency of inherited inversions was 1.4. Similar low frequencies have been reported also in two Russian populations (Kiknadze et al., 1988) and other European populations (Bovero et al., 2002). Therefore, on account of their < 1% frequency, the majority of inversions were considered endemic and rare (Caceres et al., 1997). The degree of hereditary variability of each population was estimated by dividing the number of different hereditary inversions in a population by the number of sampled larvae (H index). No relationship could be established between the H index values and the overall level of heavy metal pollution of the sediments from which larvae were collected (Sella et al., 2004). Indeed, in three populations there were almost no inherited inversions, though concentrations of some heavy metals in their sediments were among the highest. Conversely, the highest H index value was observed in a Russian population with a relatively low level of pollution (Sella et al., 2004).

Somatic Inversions

As for somatic aberrations, 184 different paracentric and five different pericentric inversions were established till date in European chironomid populations (Sella et al., 2004). Generally one somatic inversion per larva was observed. Values of the S index (i.e. the ratio of the number of different somatic inversions in a population to the number of sampled larvae) strongly differed from those of the H index of the same populations (Sella et al., 2004). The lowest values of the S index were found in populations from far less polluted sediments. Populations from the most polluted sediments had S values from six to eight times higher than those of larvae from the unpolluted basins (Sella et al., 2004).

Somatic deletion (Pompon)

Somatic aberrations were found also in the G chromosome of *C. riparius,* mainly somatic deletions affect either BRb or BRc or both. Their frequencies were highly variable. For example, the population frequency of deletion of Brb and Brc varied from .63 to 0.34 of the examined cells (Sella et al., 2004). This aberration modifies the shape of the chromosome G in a so called "pompon" chromosome. The appearance of "pompon" chromosomes has been proposed as a biomarker of heavy metal contamination in aquatic ecosystems (Michailova et al., 1998). In some cases due to trace metal pollutants G chromosome assume a very compact stage in some of the cells of an individual (Michailova et al., 2003). On the whole, in the examined populations the frequencies of different somatic inversions and chromosome G deletions show an increasing trend paralleling that of the overall heavy metal pollution. Therefore, these findings give support to the hypothesis that the increased frequency of somatic rearrangements in *C. riparius* from long term heavy metal contaminated areas may be a consequence of genomic stress induced by heavy metal pollution.

Somatic deficiency

Somatic deficiencies are generally rare in chironomids. In deficiency terminal chromosomal portions are absent from one or both chromosomal homolog. Michailova et al., (1998, 2000) shown some cases of deficiency in arm A, B, D and G from Moncalieary and Santana of Italy.

Somatic Amplification

In amplification The DNA content of chromosome or chromosomal part become higher than normal. In chironomid polytene chromosome this is manifested by heavily stained darker bands. Michailova et al., (1996) studied some specimen from Italy where two sections in arm E (A5d-g and A4f-g) and two sections in arm F (B3h and B3o) were often larger than the same sections in the standard chromosome map. They could appear in the homozygous, heterozygous and amplified homozygous states in one and the same individual. Densitometrical analysis was performed in sections A5d-g and A4f of arm E and section B3h of arm F (Sella et al., 2001). The mean value of DNA content in section EA4f was 18,8 pg., in section EA5d-g, 18.2 pg, and in section FB3h of arm F, 9.6 pg. Sections A4f and A5d-g of arm E and section FB3h of arm F had 7.13 and 15 times higher DNA contents than the lowest class in the control (Sella et al., 2001). The authors suggested that this somatic size variation was due to somatic recombination events provoked by pollutants in the sediments of water basins.

Somatic Translocation

Petrova et al., (2004) described a case of somatic heterozygous translocation of *C. riparius* from St. Petersberg population.

Conversion of euchromatin to heterochromatin

Heavy metal may also induce change of euchromatic region to heterochromatin. Chromosome AB, CD, and EF of *C. riparius* from heavy metal polluted region have shown such transformation. They have fine C heterochromatin bands that are euchromatic in samples from unpolluted region. In laboratory trial also such phenomenon has been observed. Treating samples with heavy metals induces heterochromatinization of euchromatic region where as in control no such event occurred. It has been proposed that heterochromatin had a protective role by absorbing damage and hence had a protective value (Michailova et al., 1997). In *C.riparius, C.nuditarsis* and *in C.barnensis,* another interesting event of "dark knob" formation has been observed. On chromosome G of these species the centromeric region become highly condensed to from characteristic "dark knob" in response to heavy metal toxicity (Michailova et al., 2009b).

Position effect variegation (PEV)

Another very interesting event observed in the field populations under study is position effect variegation (PEV). In PEV the activity of one gene depends on its position in the chromosome. Normally euchromatic region if placed near heterochromatic region then it also become hetero-chromatinised and in activated. Induction of PEV by ionizing radiation has been reported in Drosophila sp. In *C. riparius* high concentration of heavy metal causes inversion or translocation and as a result euchromatic region may come near heterochromatic region. As a result the euchromatic region becomes inactivated and hetero-chromatinized. Some of the somatic pericentric inversions were associated with PEV: in the homologue where the inversion is located, a pseudo puff was found, and in the other homologue the same region was in the standard condensed state (Michailova et al., 1997).

Functional Changes.

In C. *riparius* genomic stress is indicated not only by somatic structural rearrangements but also by some functional alterations in the Nucleolar Organizer (NOR) and Bambini Rings (BRs) regions.

Balbiani Rings (BR)

The Balbiani Rings are key sites of intensive transcription of genes encoding for silk proteins (Wieslander et al., 1994). These proteins are very important for Chironomids due to their participation in the construction of the tubes where larvae develop. The function of Nucleolar Organizer is essential for cellular maintenance and ribosomal production machinery, which is highly conserved through evolution (Planello et al., 2007). The response of Chironomid genomes to different stress agents is characterized by changes in puff activity of Balbiani rings (BRs) and Nucleolar Organizer (NOR). The level of puffing activity of these two very important genomic structures significantly decreased compared to the normal activity (Michailova et al., 1996, Michailova et al., 1998). Normally in *C. riparius* the puffing activity of BRc is relatively stable in fourth instars larvae and shows a fully expanded state in the prepupal stage (Diez et al., 1990) and BRb is very active in young larvae and prepupa stage, while in the middle larval stages BRb is collapsed. For instance, in some larvae from polluted stations there was a clear reversal of BRb and BRc activity in the polytene chromosomes compared to their activity in larvae from unpolluted basins. Indeed, a drastic regression of BRc was observed and a parallel expansion of BRb (Petrova et al., 2004). In other larvae both BRs could appear in slight activity or in a heterozygous state or completely repressed. Trace metals seem to induce changes in the puffing activity of *C. riparius* Balbiani rings which are similar to those induced by heat shock or sugar feeding (Yagi, 1984). The same tendency has been observed also in other Chironomid species (Michailova et al., 2009a, Michailova et al., 2009b). These findings suggest that the BR transcription mechanism can react in a similar way to different stress situations. The effect of copper on the activity of Balbiani rings (BR1 and BR2) and nucleolar organizing region (NOR) in chromosome IV of the salivary gland of the 4th instar larvae of *Chironomus ninevah* has been investigated by Aziz et al., (1991). They showed that sublethal concentrations, i.e. 0.02, 0.1, 0.15 and 0.2 mg per liter suppress the activity of BR1. The same concentrations reduced the activity of BR2 and NOR.

Puffing

Michailova et al., (2009a) described an interesting phenomenon in *C. acidophilus* from Afon Goch river of UK. Here in chromosome G one of the BRs was not expressed. But at the same time the telomere region had swelled into a puff. The authors described it as compensatory puffing. In *C. riparius* an induction of a puff in chromosome G (Dc, E2de) was detected, in either a homozygous or heterozygous state (Michailova et al., 1998).

Nuclear Organising Region (NOR)

Nucleolar Organizer also showed various changes in its activity, from a very high activity of both homologues to a heterozygous state, or a slight activity or a complete collapse (Petrova et al., 2004). Intermediate activity was recorded very often to show that ribosomal genes and the Nucleolar function are direct targets for cadmium toxicity (Planello et al., 2007). Inter specific difference in respect to functional changes in response to heavy metal have been noted. *C. riparius* show response mainly by structural changes where as *C. piger* show functional changes. The functional changes in *C. piger* include already mentioned altered activity of BR and NOR and also appearance of unknown puffing in the telomeric region (Michailova et al., 2004).

Ectopic pairing and asynapsis

In few cells of the polytene chromosomes of *G. glaucus* (Ilkova, 2004), and of *C. acidophilus* (Michailova et al., 2009a), and of *C. riparius* (Michailova et al., 1996, 2000), an ectopic pairing between different chromosomes was detected. For instance, in *C. riprius*, an ectopic conjugation was observed between chromosome G, section Ee and telomeres A, C, D, E and F (Michailova et al., 1996). The lowest degree of contacts was noticed between centromeres AB, EF and G chromosomes. Normally in *C. riparius* the homologues of chromosome G are paired. However, material cases of disturbed pairing were observed in some populations studied or exposed: either both homologues were completely asynapted or they were asynapted in a specific site of the chromosome. The frequency of asynapsis increased from section A to E (Michailova et al., 1996, 1998, 2003).

Relation between heavy metal concentration and frequency of polymorphism

Sarkar et al., (2011a) studied the effect of arsenic on chromosomes of Chironomus striatipennis collected from West Bengal, India. Both collected larvae from Arsenic polluted water body and laboratory treated larvae show several types of aberration like bleb formation, asynapsis and heterozygous inversion. Asynapsis again appeared to be of different forms, such as short terminal, intercalary and long major type. Induction tests in the laboratory showed that not only did morphological deformities appear in the larvae, but also aberrant forms of polytene chromosomes appeared in the treated larvae. In fact the treated larvae in the laboratory were more affected than the naturally occurring larvae. In the laboratory test it has been shown that with increasing concentration of As_2O_3 from 2 mg/ kg to 8 mg/kg of soil-sediment the frequency of aberration in every chromosome increase 2-

2.5 times. It is also noteworthy that in most of the studies, out of the four chromosomes the IVth chromosome that is the G arm is the most frequently affected one. Michailova et al., (1998, 2009) have shown deletion and hetero-chromatinization can cause “pompon” and “dark knob” on arm G. Sarkar et al., (2011b) noted another nine aberrant structural changes in it. They grouped it into three main types viz. apparently normal with minor inversion, the aberrant looped form with heterozygous inversion, and asynaptic form. Apart from this there are aberrant Balbiani Rings and puffing as noted above. All these events show that the fourth small chromosome is the most responsive to the stress of heavy metal pollution.

Possible mechanism of chromosomal aberration due to heavy metal pollutants

Oxidative stress

Recent studies indicate that heavy metal act as catalysts in the oxidative reaction of biological macro molecules and therefore much of their toxicities are due to the oxidative tissue damage. Redox active metals such as iron, copper, chromium undergo redox cycling whereas redox inactive metals such as lead, cadmium and mercury deplete cells major antioxidants particularly thiol containing antioxidants and enzymes. So both these two type of metals contribute into the increase of production of reactive oxygen species (ROS) such as hydroxyl radical (OH*), superoxide radical (O_2*-) or hydrogen peroxide (H_2O_2). Enhanced generation of ROS can overwhelm the cells anti oxidative defenses and can result in a condition called oxidative stress. Oxidative stress can affect variety of macro molecules one of which are DNA and could lead to the mutation or strand breakage (Ercal et al., 2001).

Repetitive DNA and chromosomal aberration.

There are two types of DNA sequences found in the genome: Obligatory sequences and facultative sequences. Obligatory sequences are made up of unique sequences and constitutive structural genes; whereas the facultative part consists of mainly repetitive DNAs and transposable genetic elements. Three such repetitive DNA elements have been localized on the chironomid chromosomes and their sites have been compared with common breakpoints of those chromosomes. Here breakpoints means are the sites where these chromosomes often break during chromosomal rearrangement due to different stress factor such as heavy metals. The repeated DNA elements are Alu and Hinf and one retrotransposon NLRCth1. In *C. riparius* and in *C. piger* they show characteristic localization on different sites of the chromosome by which the species could be distinguished by using them as molecular marker. The genome of the phylogenetically younger species, *C. riparius*, is rich in these elements in comparison with the older *C. piger* species. This higher distribution of repetitive sites could be correlated with the distribution of breakpoints of chromosomal rearrangements. Zampicinini et al., (2004) performed PCR-based Transposon Insertion Display (TID) and found a high level of insertion variability in *C. riaprus*. The greater number of copies of dispersed repetitive sequences in the *C. riparius* genome than in *C. piger* determines the higher sensitivity of the *C. riparius* genome, where somatic aberrations affected the whole chromosome, whereas in *C. piger* they are concentrated mainly in pericentromeric regions and in few sites of arms D and F (Michailova et al., 2009c). Thus, both species, which differ in DNA organization (*Chironomus riparius* has a greater content of DNA elements rich in repetitive DNA elements, dispersed among all chromosomes.) respond to stress agents in different ways. None or significantly less chromosome rearrangements (inversions) were found in *C. piger* (Michailova et al., 2009c).

However, it was proven that the significant association formed between breakpoints and sites of distributions of NLRCth1, Alu, and Hinf clusters in the genomes of both species (Ilkova et al., 2007). For instance, twenty of the total number of weak points (58.8 %) occurred in the vicinity of bands hybridizing to probes of Alu and Hinf satellite DNA (Bovero et al., 2002). Such correlations were found also for the distribution of CRTR clusters and breakpoints of rearrangements in *C. piger* (Michailova et al., 2009c). Location of the pCthC12HR transposable element (TE) in the genome of *C. riparius* was studied by Kiknadze et al., (1987) in larvae of the Novosibirsk population, and coincidences between the location of this element and sites of chromosomal breaks were detected. The data about the sensitivity of the genome to stress agents confirm the idea put forward by Bovero et al., (2002) that repetitive DNA and TE-rich regions are more sensitive to genotoxic agents and that they are potentially prone to rearrangements.

Conclusion

Organism and environment interact with each other in much level: from community to the genetic and molecular. In Chironomid larvae the manifestation of this interaction is evident in the cytogenetic level. The large polytene chromosome act as a mirror of the events at the DNA level and every molecular change is reflected in the morphology of the large chromosome. So this polytene system can be used as indicators of genotoxic concentration of pollutants in aquatic ecosystem. The changes in the system are species specific and also concentration specific – either in the structural or in the functional level. In some cases specific alteration may be created by specific heavy metal. Also the breakage of the chromosome is non random; the chromosomes have specific break point and these breakpoints are closely associated with repetitive DNA and transposable elements.

So each change in chromosome can be analysed in respect to several factors and can be used as a powerful tool in providing early warning signals of adverse long term effects of toxic agents at the individual, population and community level. The mystery lies in the fact how the minute organism manages to combat the unwanted environment with so many heavy metals in so much higher concentration. From all the findings several questions 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. More research attempt in future will be required to answer all these questions.

References

Alam, M. G. M., Snow, E.T. and Tanaka, A. (2003). Arsenic and heavy-metal contamination of vegetables grown in Samta village Bangladesh. *Sci. Total E nviron*. 308: 83–96.

Aziz, J. B., Akrawi, N. M. and Nassori, G. A. (1991). The effect of chronic toxicity of copper on the activity of Balbiani rings and nucleolar organizing region in the salivary gland chromosomes of

Chironomus ninevah larvae. *Environmental Pollution.* 69(2-3): 125-130.

Beldi, H., Gimbert, F., Maas, S., Scheifler, R. and Soltani, N. (2006). Seasonal variations of Cd, Cu, Pb and Zn in the edible Mollusc Donax trunculus (Mollusca, Bivalvia) from the gulf of Annaba, Algeria. *Afr. J. Agric. Res*. 1(4): 85-90.

Benke, A. C. and Wallace, J. B. (1997). Trophic basis of production among riverine caddisflies: implications for food web analysis. *Ecology.* 78: 1132-1145.

Bovero, S., Hankeln, T., Michailova, P., Schmidt, E. and Sella, G. (2002). Nonrandom chromosomal distribution of spontaneous breakpoints and satellite DNA clusters in two geographically distant populations of *Chironomus riparius* (Diptera, Chironomidae). *Genetica.* 115: 273-281.

Brus, D., Li, Z.B., Temminghoffd, E.J.M., Song, J., Koopmans, G.F., Luo, Y.M. and Japenga, J. (2009). Predictions of spatially averaged cadmium cContents in rice grains in the Fuyang Valley, P.R. China. *J. Environ. Qual*. 38: 1126-1136.

Burger, J. (2006). Bioindicators: A Review of Their Use in the Environmental Literature 1970–2005. *Environmental Bioindicators*. 1(2): 136-144.

Caceres, M., Barbadilla, A. and Ruiz, A. (1997). Inversion length and breakpoint distribution in the Drosophila buzzatii species complex: Is inversion length a selected trait? *Evolution*. 51: 1149-1155.

Chakraborti, D., Das, B. and Rahman, M. M. (2009). Status of groundwater arsenic contamination in the state of West Bengal, India: a 20-year study report. *Mol. Nutr. Food Res.* 53: 542–551.

Cranston, P. S. (1995). Introduction. In: The Chironomidae: The Biology and Ecology of Non- Biting Midges, P. D. Armittage, P. S. Cranston, and L. C. V. Pinder (Eds). Chapman and Hall, New York. Pp. 1-6.

Devai, G., Miskolczi, M. and Wülker, W. (1989). Standardization of chromosome arms B, C and D in *Chironomus* (Diptera: Chironomidae). *Acta. Biologica. Debrecina. Supplementum. Oecologica Hungarica.* 2: 79-92.

Diez, L., Cortes E., Merimo, J. and Santa Cruz, M. (1990). Galactose induced puffing changes in *Chironomus thummi* Balbiani rings and their dependence on protein synthesis. *Chromosoma.* 99: 61-70.

Dube, M. G. and Culp, J. M. (1996). Growth responses of periphyton and chironomids exposed to biologically treated bleached-kraft pulp mill effluent. *Environmental Toxicology and Chemistry.* 15: 2019-2027.

Ercal, N., Gurer-Orhan, H. and Aykin-Burns, N. (2001). Toxic metals and oxidative stress Part I: Mechanism involved in metal induced oxidative damage. *Current Topics in Medicinal Chemistry.* 1: 529-539.

Farlex Incorporated. (2005). Definition: Environment, *The Free Dictionary,* (2005) Farlex Inc. Publishing, U.S.A. (www.thefreedictionary.com/).

Haefliger, P., Mathieu-Nolf, M., Lociciro, S., Ndiaye, C., Coly, M., Diouf, A., Lam Faye, A., Sow, A., Tempowski, J., Pronczuk, J., Filipe, A. P., Bertollini, R. and Neira, M. (2009). Mass Lead Intoxication from Informal Used Lead-Acid Battery Recycling in Dakar, Senegal. *Environ Health Perspect*. 117: 1535-1540.

Hägele, K. (1970). DNA replicationmuster der speicheldrusen chromosomen von

Chironomiden *Chromosoma.* 31: 91–138.

Hutton, M. and Symon, C. (1986). The Quantities of Cadmium, Lead, Mercury and Arsenic Entering the U.K. Environment from Human Activities. *Sci. Total Environ*. 57: 129-150.

Ilkova, J. (2004). Chromosome variability of species of sub-family Chironominae (Diptera, Chironomidae) from Polluted Regions near Sofia. *Acta Zool. Bulgaria*. 56(1): 15–30.

Keyl, H.G. (1962). Chromosomen evolution bei Chironomus.II. hromosomenumbauten undphylogenetische Beziehungen der Arten. *Chromosoma.* 13: 464-514.

Kiknadze, I. (1987). Chromosomal polymorphism in natural pop-ulations of plumosus species-group of West Siberia. *Entomol. Scand. Su: l.* 29: 113–121.

Kiknadze, I., Gunderina, L., Filipova, M. and Seray, E. (1988). Chromosomal polymorphism in natural and laboratory populations of *Chironomus thummi* Kieff. *Genetika*. 24: 795-1805 (In Russian).

Kiknadze, I.I., Shilova A. and Kekris, I. (1991). Karyotype and morphology of larvae in Chironomini. *Atlas. Novosibir*sk. Pp. 117.

Lenntech Water Treatment and Air Purification (2004). Water Treatment, Published by Lenntech, Rotterdamseweg, Netherlands (www.excelwater.com/thp/filters/Water-Purification.htm.)

Martin, J., Chung, H., Balakrishnan, T. & Robin, C. (2010). Preliminary Physical Maps of the *Chironomus* Genome, with a Focus on Genes Potentially Involved in Response to Heavy Metals. In: Ferrington, L. C., Jr. (ed.). *Proceedings of the XV International Symposium on Chironomidae*. Chironomidae Research Group, University of Minnesota, Saint Paul, Minnesota. Pp. 222-234.

Martinez, E. A., Moore, B. C., Schaumloffel, J. and Dasgupta, N. (2001). Induction of morphological deformities in Chironomus tentans exposed to zinc- and lead-spiked sediments. *Environmental Toxicology and Chemistry.* 20: 2475-2481.

Maximova, F. L. (1976). The karyotype of Chironomus plumosus from Ust- Izhola wild population of Leningrad region. *Tsitologya.* 18: 1264-1269.

Meester, L. D. (1993). Genotype, Fish-Mediated Chemical, and Phototactic Behavior in *Daphnia Magna*. *Ecology*. 74: 1467–1474.

Michailova, P. (1985). Method of breeding the species from family Chironomidae, Diptera in experimental conditions CR. *Acad. Bulg. Des Sciences.* 38(9): 1179–1181.

Michailova, P. and Petrova, N. (2004). Natural hybridization in Insects, Diptera (Model group–family Chironomidae). In *Evolution and Ecology*, Sofia, U. S. Bulgaria. Pp. 9-19.

Michailova, P. Petrova, N., Ramella, L., Sella, G., Todorova, J. and Zelano, V. (1996). Cytogenetic characteristics of a population of *Chironomus riparius* Meigen 1804 (Diptera, hironomidae) from a polluted Po river station. *Genetica.* 98: 161-178.

Michailova, P., Ilkova, I. and White, K. N. (2003). Functional and struc-tural rearrangements of salivary gland chromosomes of Chironomus riparius Mg. (Diptera, Chironomidae) in response to freshly neutralized aluminium . *Envi-ron. Pollut.* 123: 193–

207.

Michailova, P., Ilkova, J., Kerr, R. and White, K. (2009a). Chromosome variability in *Chironomus acidophilus* Keyl from the Afon Goach, UK – a river subject to long-term metal pollution. *Aquatic Insects.* 31(3): 213–225.

Michailova, P., Ilkova, J.and Hankeln, T. (2009c). Somatic break-points, distribution of repetative DNA and non-LTR retrotransposones insertion sites in the chromosomes of *Chironomus piger* Strenzke (Diptera, Chironomi-dae). *Genetica.* 135: 137–148.

Michailova, P., Petrova, N., Sella, G., Ramella, L. and Bovero, S. (1998). Structural,functional rearrangements in chromosome G in *Chironomus ripari*us Meigen (Diptera, Chironomidae) collected from a heavy metal polluted Piedmont station, Italy. *Environmental Pollution*. 103: 127–135.

Michailova, P., Ramella, L., Sella, G.and Bovero, S. (1997). C-band variation in polytene chromosomes of *Chironomus riparius* Mg (Diptera, Chironomidae) from a pol-luted Piedmont station (Italy) *Cytobios.* 90: 139–151.

Michailova, P., Szarek-Gwiazda, E. and Kovnacki, A. (2009b). Effect of contaminants on the genome of some species of genus *Chironomus* (Chironomidae, Diptera) live in sediments of Dunajec river and Czorsztyn re-servoir. *Water, Air and Soil Pollut.* 202: 245–258.

Michailova, P. and Mettenen, A. (2000). Cytotaxonomical variabil-ity of *Chironomus plumosus* L. and *Chironomus anthracinus* Zett. (Diptera, Chironomidae) fromindustrial and municipal polluted areas of Finland *Caryologia*. 53(1): 69–81.

Miller, J.R., Hudson-Edwards, K. A., Lechler, P. J., Preston, D. and Macklin, M. G. (2004). Heavy-metal contamination of water, soil and produce within riverine communities of the Rıo Pilcomayo basin, Bolivia. *Sci. Total Environ*. 320: 189–209.

Mitra, A., Bannerjee, K., Ghosh, R. and Ray, S. K. (2010). Bioaccumulation pattern of heavy metals in the shrimps of the lower stretch of the River Ganga. *Mesopot. J. Mar. Sci.* 25 (2): 1 - 14.

Oliver, D. R. (1971). Life history of Chironomidae. *Annual Review of Entomology.* 16: 211- 230.

Onder, S., Dursun, S., Gezgin, S. and Demirbas, A. (2007). Determination of Heavy Metal Pollution in Grass and Soil of City Centre Green Areas (Konya, Turkey). *Polish J. of Environ. Stud*. 16(1): 145-154.

Pandey, J., Subhasis, K. and Pandey, R. (2009). Metal Contamination of Ganga River (India) as Influencedby Atmospheric Deposition. *Bull Environ Contam Toxicol*. 83: 204–209.

Petrova, N. and Michailova, P. (2002). Cytogenetic characteristics of *Chironomus bernensis* Wülker and Klötzli (Diptera, Chironomidae) from a heavy metal polluted station in Northern Italy. *Ann. Zool.* 52(2): 227–233.

Petrova, N. A., Michailova, P. and Ilkova J. (2004). Comparative Cytogenetic Variation of the Salivary Gland Polytene Chromosomes in *Chironomus riparius* Mg. 1804 (Diptera, Chironomidae) from two Polluted Biotopes of Bulgaria and Russia, *Russian Journal of Genetics*. 40(1): 40 – 48.

Planello, R., Martinez-Guitarte L. and Morcillo, G. (2007). Ribosomal genes as early target of cadmium,induced toxicity in *Chironomus* riparius larvae. *Sciences in the Total Environment*. 373: 113 – 121.

Saha, S. B., Mitra, A., Bhattacharya, S. B., and Choudhury, A. (2001). Status of sediment with special reference to heavy metal pollution of a brackishwater tidal ecosystem in northern Sundarbans of West Bengal. *Tropical Ecology*. 42(1): 127-132.

Sarkar, P., Bhaduri, S., Ghosh, C. and Midya, T. (2011a). The species of chironomus as biosensor in detecting environmental pollution: a study on chironomus striatipennis kieffer (diptera: chironomidae). *The Ecoscan,* special issue. (1): 363-368.

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

Sella, G., Bovero, S., Ginepro, M., Michailova, P., Petrova, N., Robotti, C. A. and Zelano, V. (2004). Inherited and somatic cytogenetic variability in Palearctic populations of *Chironomus riparius* Meigen 1804 (Diptera, Chironomidae). *Genome.* 47: 332–344.

Sella, G., Robotti, C., Michailova, P. and Ramella, L. (2001). Repetitive DNA size variation in three sections of the chromosome EF in a population of *Chironomus riparius* Mg (Diptera, Chironomidae) from Piedmont (Italy). *Caryologia*, (Firenze). 54(2): 155–160.

Temara, A., Skei, J. M., Gillan, D., Warnau, M., Jangoux, M. and Dubois, P. (1998). Validation of the asteroid Asterias rubens (Echinodermata) as a bioindicator of spatial and temporal trends of Pb, Cd, and Zn contamination in the field. *Mar. Environ. Res*. 45(4-5): 341-356.

Timmermans, K. R., Peeters, W. and Tonkes, M. (1992). Cadmium, zinc, lead and copper in *Chironomus riparius* (Meigen) larvae (Diptera, Chironomidae) - Uptake and effects. *Hydrobiologia.* 241: 119-134.

Wieslander, L. (1994). The Balbiani ring multigene family: coding sequences and evolution of a tissue, specific function. *Proc. Nucleic Acids Res*. 48: 275-313.

Yagi, S. (1984). Effects of ligation on ethanol induced Balbiani ring puffing in salivary gland of *Chironomus*. *Chromosoma*. 89: 274 – 279.

Yingmei, Z., Yejing, W., Runliu, Y. U., Sheng, Z. and Zhenbin, W. U. (2008). Effects of heavy metals Cd2+, Pb2+ and Zn2+ on DNA damage of loach *Misgurnus anguillicaudatus*. *Front. Biol.* 3(1): 50-54.

Zampicinini, G. P., Blinov, A., Cervella, P. and Sella, G. (2004). Insertional polymorphism of a non – LTR mobile element (NLRCth1) in European populations of *Chironomus riparius* (Diptera, Chironomidae) as detected by transposon insertion display. *Genome.* 47(11): 54–1163.