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Chromosomal rearrangements in *Chironomus sp.* as genosensors for monitoring environmental pollution

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Abstract

The main objective of our research was to use the polytene system existent in the larvae stage of the common Diptera species of Chironomus as a tool for identifying genomic sensors in evaluating the environmental pollution degree. We analysed polytene chromosomes of larvae of two populations from two polluted regions: Bucharest (collected from some pools of Botanical Garden) and from Mureş Valley, Western area of Romania. 627 cells from 55 larvae of the Bucharest population and 352 cells from 30 larvae of the Mures Valley were analyzed for the chromosomal aberrations and were processed using both acetocarmine staining method and DAPI staining for polytene chromosome squashes. In both populations a large number of somatic chromosomal rearrangements of their polytene chromosomes were encountered. The changes in the puffing pattern were also considered. Since a large array of different chromosomal rearrangement was evidenced in these two analysed populations we concluded that Chironomus chromosomal polytene complement might be considered as an ideal tool in searching for the genomic sensors used in evaluation of environmental pollution degree. All the rearrangements identified in Chironomus sp polytene chromosomal complement can be considered as biomarkers which provide early warning signals of the adverse long term genotoxic effects of the pollution in different other organisms.

Keywords: *Chironomus,* genomic sensor, polytene chromosomes, chromosomal aberrations, pollution

Introduction

The standard karyological characteristics of the polytene chromosomes of some species can be used as a basis for revealing chromosomal aberrations and changes in aspect of heterochromatin and functional activity under the action of environmental mutagens.

The micro morphology of polytene chromosomes has provided many key traits for dipteran's species characterization and phylogenetic investigations. The study of the chromosomal aberrations in polytene systems offers multiple advantages. First, any structural rearrangement is expressed in a correspondent alteration in somatic synapses. Second, the puffing phenomenon represents a morphological sign of gene activations. Third, differential replication takes place in polytene chromosomes.

Thus, the polytene system becomes an excellent study system of the chromosomal aberrations, inaugurated in the middle of the 20th century through the glorious research of the famous Russian born American evolutionary geneticist Theodosius Dobzhansky, whose research have contributed to the substantiation of the evolution synthetic theory and to the validation of its paradigms with genetics data obtained through field and lab investigations.

His classical contributions to population genetics was performed in *Drosophila pseudoobscura* and *D. persimilis* in which he identified different translocations that conferred various adaptive values with respect to temperature variation to the fruit fly population. [1]

Chironomids are a widely distributed and abundant group of Dipteran's species in freshwater ecosystems. Their larval stages are the most metabolically active of their life and, as a consequence, also the most critical, responsive to environmental stress and prone to undergo different damages under the effects of pollutants. Chironomide larvae are prospective subjects for cytogenetic monitoring due to their polytene chromosomes which have a large size and an obvious banding pattern. [2]

In *Chironomus sp* chromosomes structure and rearrangements most exciting and provocative work was performed by W. Beermann (1951-1954), a famous representative of German modern Cytology. He was the first to identify an inversion distinguishing two species of *Chironomus, C. pallidivitatus* and *Camptochironomus tentans*. [3]

Due to the presence of polytene chromosomes with highly conserved banding patterns, it is possible to recognize homologous regions of chromosomes in karyotypes of all *Chironomus* species. However, when paracentric inversions have occurred in different chromosomal arms, during speciation, the divergence of chromosomal banding sequences have already taken place.

For *Chironomus*, a Bulgarian, Russian and Italian international team has studied over several years the changes which appeared at the polytene system level from the salivary glands of the *Chironomus* larvae, which develops in heavy metals (Pb, Cu) polluted areas, detecting the presence of numerous inversions, centromere breakages, telomere condensations/decondensations, amplifications of some polytene areas, desynapses – alteration in puffing pattern etc.

Philinkova (2007) studied species composition and chromosomal polymorphism in three natural populations of *C. plumosus* Linnaeus from the South TransUral region and has detected six inversion disk sequences in five out of seven chromosome arms; the number of disk sequences varied in different arms: 2 – in arm A, 3 – in arm B, 2 – in arm D, 2 –in arm E, and 1 – in arms F and G respectively. *C. plumosus* has the only type of heterozygous inversion in arm A, but it clearly prevails (26.7-35%) in comparison with heterozygous combinations in other arms. Larvae of this species have two types of heterozygous inversions in arm B and single types of heterozygous inversions in arms D and E, whereas examined populations of the same species have monomorphic arms F and G. [4]

An average number of heterozygous inversions per specimen is equal to 0.95 for populations of *C. plumosus* in the Palaearctic region. [5]

The study of chromosomal polymorphism in natural populations of animals allows us to evaluate the cytogenetic differentiation of populations, to determine the role of fixed chromosomal rearrangements in speciation, and to reconstruct the cytogenetic history of species and their patterns of migration. Chromosomal polymorphisms showed geographical and/or temporal variability. The midge *C. plumosus* L. is a good candidate for such analysis because it is widely distributed and is frequently sampled as a biological indicator of lake eutrophy. No longitudinal, latitudinal, or altitudinal clines of inversion polymorphism are known in *C. plumosus*, in contrast with dipteran species such as *D. melanogaster* and *D. subobscura*, which have clear-cut clines for several inversion sequences[6]. In addition to inversion polymorphism, genomic polymorphism occurs in Palearctic *C. plumosus* populations. This is associated with the presence of additional B chromosomes which represent genomic polymorphism and with the appearance of triploids [7, 8]. Usually there is one polytene B chromosome per nucleus and correspondingly, there are two additional

chromosomes on mitotic plates in mitotically dividing cells. These polytene B chromosomes lack a clear banding pattern. Heterozygosity of a centromeric band was observed in many populations, with frequencies from 2 to 51% [9]. The level of chromosomal polymorphism (i.e., number of sequences, percent heterozygosity) is much lower in peripheral populations of polymorphic species [6].

Starting from this work we aim to evidence the potential chromosomal aberrations appeared in the polytene chromosome structures of *Chironomus spercies* after the exposure to different polluting agents (radionuclids, heavy metals etc.) using classical cytogenetics techniques (acetocarmin staining and the Feulgen method) and fluorescence methods (*DAPI*) on the salivary gland cells.

Material and methods

We analyzed polytene chromosomes of larvae of two *Chironomus* populations from two polluted regions: Bucharest (collected from some pools of Botanical Garden) affected by pollutant emissions of a thermo-electric power station and from water resources from Mures Valley, Western area of Romania – uranium mines, in which heavy metal and radionuclids from traces were detected. Our results were compared to a standard karyotype from a natural population described by Golygina et al., 2007 [10].

Methods

Evidencing the polytene complement using the acetocarmin staining: extracting the salivary glands in dye solution – acetocarmin; acetocarmin staining, squashing; visualization at Olympus microscope – chromosomal analysis was performed on fresh preparations; chromosomes with an average rate of polyteny were selected;

Evidencing the polytene complement using fluorescent agents staining – DAPI : extraction of the salivary glands in KCl hypotone solution; solution removal and material fastening with 45% acetic acid aquatic solution for one minute; *DAPI* solution addition – 30 minutes staining; slide washing and material squashing; visualization under an Olympus microscope – chromosomal analysis was performed on fresh preparations, and chromosomes with an average rate of polyteny were selected;

627 cells from 55 larvae of the Bucharest population and 352 cells from 30 larvae of the Mures Valley were analyzed for the chromosomal aberrations and were processed using both Feulgen method and DAPI staining for polytene chromosome squashes.

Results and discussions

This is a preliminary study in which we focused on identification of some conclusive chromosomal markers that can be used in evaluating the pollution effects at chromosomal level.

The polytene chromosomes have their origins in the chromosomes of the normal chromosomal complement from the somatic cells, which divides mitotically up to one point, when it enters an endoreduplication cycle. The result of the endoreduplication is the genesis of a high number of chromatids, which remains in a parallel arrangement, forming a polytene structure. Parallel with the polytenization, the union of the centromeres of all chromosomes in a single large heterochromatic structure called chromocenter, takes place. The band and interband patterns of the polytene chromosomes are highly reproductive and characteristic for

a certain species. Any change in the structure of these chromosomes which leads to a certain non-homology (i.e. inversions and duplications) is expressed obviously at the arms of the giant chromosome level through the appearance of some alterations of the somatic pairing, expressed as loops. At the level of these loops, the polytene chromosomes could not pair any longer, thus revealing the duplicate character (paired, synapsed) of each chromosomal arm, excepting the X chromosome, from the male larvae.

In *Chironomus*, the polytenization process lacks the stage of the chromocenter formation. In polytene chromosomes of *Chironomus* the chromosome entities are independent; each homologous chromosome synapses with its partner in a highly specific mechanism pairing, after which the paired homologous chromosomes suffer the polytenization process (Fig. 1).

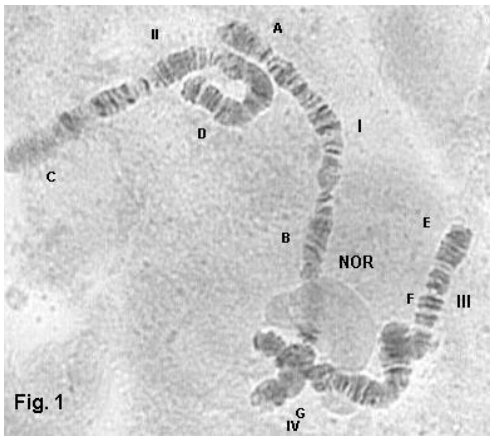

Figure 1. *Chironomus* sp. polytene complement and NOR (60x)

Most species of *Chironomus* have a diploid chromosome number of 2n = 8 with two pairs of metacentric chromosomes, which are the biggest ones, one pair of submetacentric chromosomes and one pair of short telocentric chromosomes, the last one presenting *nucleolus organizing region (NOR)*. In Chironomus, the nucleolus-organizing activity is a major part of polytene chromosomal phenotype. The first three chromosomes pairs of *Chironomus plumosus* complement represent some 90% of *Chironomus* genome. The level of polyteny in this species appears to be extreme in some cells of the salivary glands. In the salivary glands the chromosomal homologues are tightly synapsed, making it easy to recognize rearrangements. A thick band of centromeric heterochromatin divides each meta/submetacentric chromosome into two easily discernible arms. The homologues are numbered I-IV in order of decreasing length, and the chromosomal arms are designated A-G. Chromosomes I-II are metacentric with arms A + B, C + D, chromosomes III are submetacentric with arms E + F; chromosome IV is telocentric and contains only arm G. Thus, all species have seven chromosomal polytene arms. Chromosomes were divided into numbered segments at recognizable bands for the purpose of designating inverted regions. The karyotype of *C. plumosus* is very polymorphic in some populations with all chromosomal arms except arm G having alternative inversion sequences. Arm G, which bears the nucleolus and two Balbiani rings, is monomorphic. A third Balbiani ring is located very near the nucleolus organizing region and has only been identified by electron microscopy [11]. The homologues of G are usually not paired.

In both investigated populations a large number of polytene chromosomal rearrangements were identified. No specimen was found with a standard karyotype (i.e. free at all of chromosomal aberrations). The most frequent changes found in the polytene system of

the analyzed material were: inversion loops and/or desynapsis loops, chromosomal translocations, chromosomal circularization and puffing pattern alteration (Figs. 2 -5).

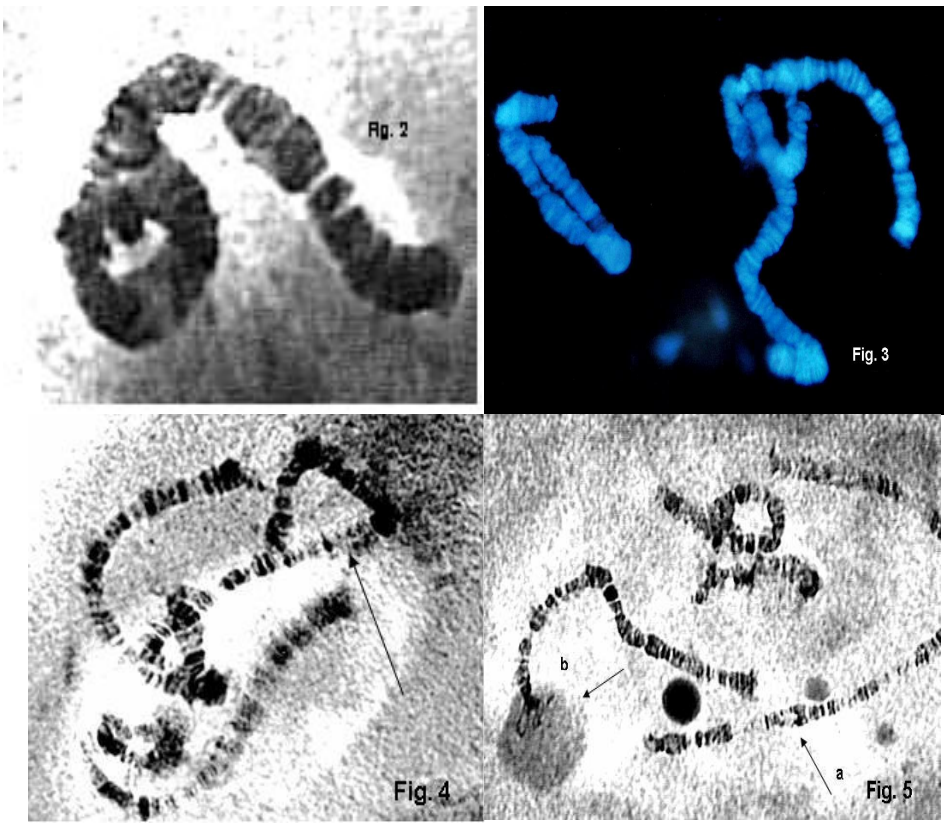

Figure2. Telomere circularization in *Chironomus plumosus* (120x) **Figure 3.** Telomere circularization in *Chironomus plumosus*, with the translocation of a chromosomal fragment or of a B chromosome (DAPI stained) (60x) **Figure 4.** Desynapsis loops in *Chironomus plumosus* (60x) **Figure 5.** Translocation of NO region on chromosome II, inversions loop in chromosome I in *Chironomus plumosus* (60x)

The telomeres are heterochromatic to the majority of *Dipterans*. They are composed of typical α-heterochromatin and like centromores are attached to the inner membrane of nuclear envelope [12]. In *Chironomus*, the telomeres have a well defined structure, fan alike (Fig. 6). The telomere structure can be modified by different exogenous factors.

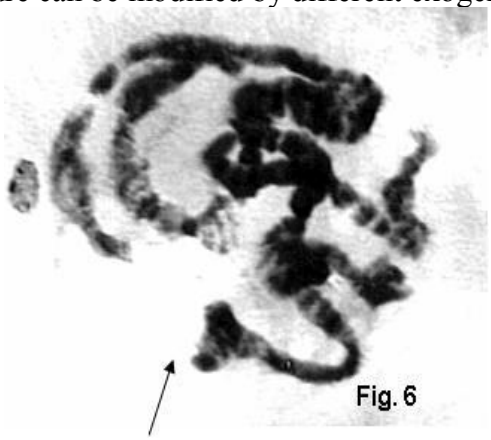

Figure 6. Fan-like telomere structure in *Chironomus plumosus* (60x)

Another chromosomal marker which can be considered in such studies can be the puffing process – a chromosomal phenotype phenomenon connected with gene activation. The puffing represents a local decondensation of nucleohistonic elements. Some puffs can be very large, becoming Balbiani rings (fig. 7).

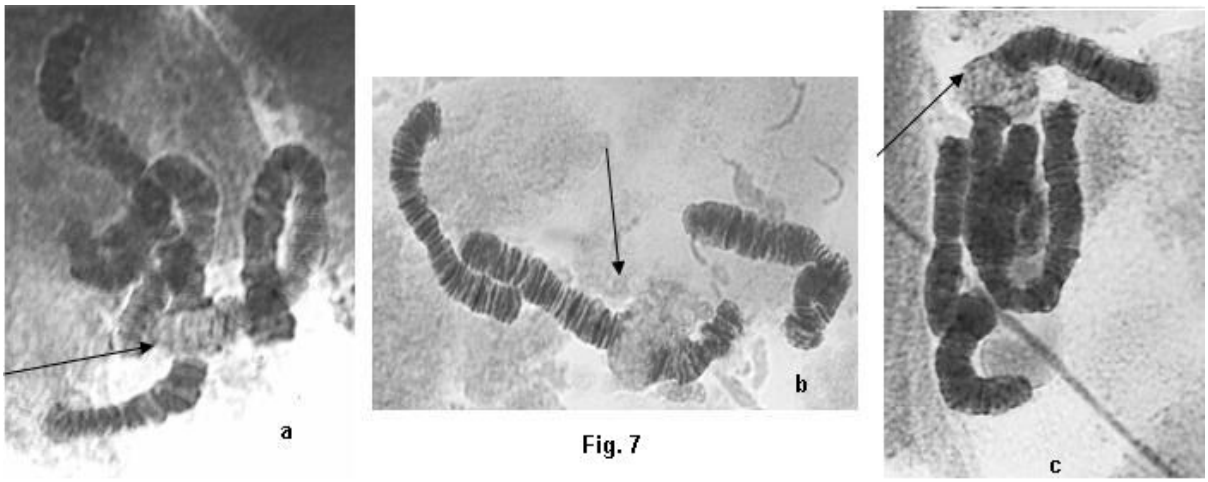

Figure 7. Different Balbiani rings on chromosome I, revealing gene activation timing (a,b and c) in *Chironomus plumosus* (100x)

The most expressive puffing process we have encountered in Chironomus is the puffing of nucleolus-organizing region of polytene chromosome IVth that leads to the appearance of a pair of nucleoli, one for each homologous chromosome. Changes observed in a functional activity of the 4th chromosome were very interesting. In natural population on this chromosome a system of three Balbiani rings BRa(No), BRb and BRc is located [11, 13, 10]. BRs are permanently active puffs which encode the major polypeptides of the salivary glands. Generally in an unpolluted ecosystem the three BRs are almost identical in size. In contrast, in both studied populations a drastic regression of BRc, simultaneously with the expansion of BRa (No) was frequently observed. Apparently the BRs transcription mechanism reacts in the same way to different stressful situations. (fig. 8)

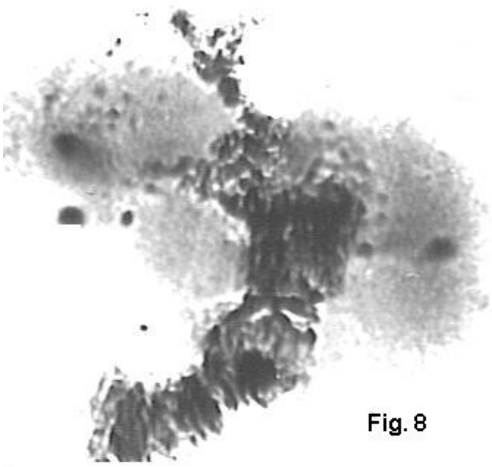

Figure 8. Expansion of a BRa(No) from chromosome IVwhich function as a nucleolus –organizing region revealing its duplicated nature in *Chironomus plumosus* (200x)

In connection with chromosomal rearrangements, we will look for identification of a correlation between chromosomal rearrangement in itself and the puffing phenomenon, which can be interpreted in the terms of activation of some genes due to establishing of new spatial neighbouring between heterochromatic and euchromatic domains of the chromosomes.

The rapid changes in gene expression pattern in any cell in response to environmental damage (e.g. supraoptimal temperatures) reveal the existence of an universal and conserved cell mechanism which works from prokaryotic to eukaryotic cells. It is known as the heat shock response. A group of genes, named *heat shock (HS)* genes, is activated upon heat shock. Subsequently, the cells carry out an efficient and massive synthesis of the so-called heat shock proteins, which account for the physiological and protective role of the response which is not fully understood yet.

The heat shock response has been described in some *Chironomus* species. A set of loci became puffed and actively transcribed. [14, 15, 16] The most striking feature is the puffing of telomere from chromosome IIIR (chromosome F).

We have found the same remarkable telomere puffing in Mures Valley population of *C. plumosus*, by *DAPI* staining. (fig. 9)

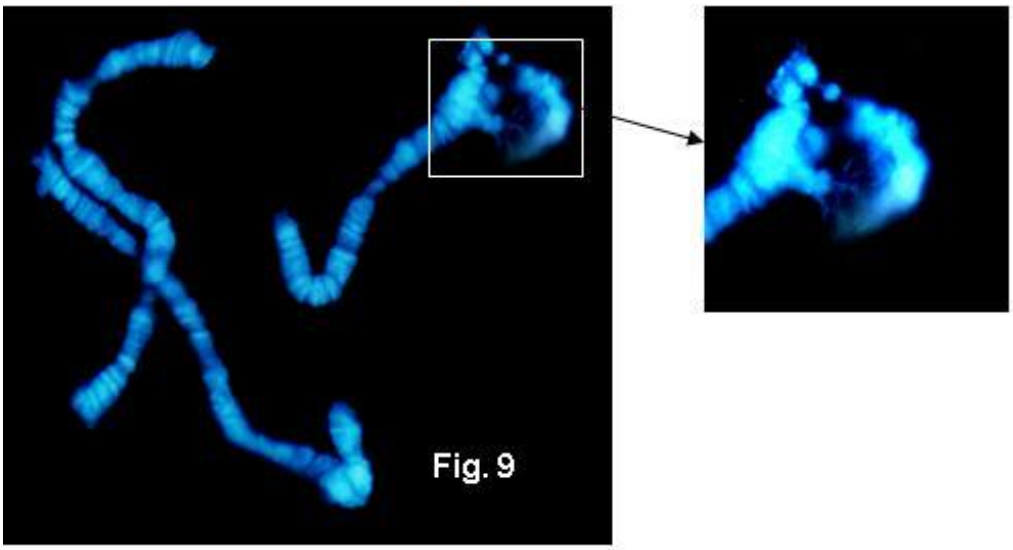

Figure 9. Telomere puffing in chromosome III in *Chironomus plumosus* (DAPI stained) revealing a possible HS-like gene activation as a response to the environmental stress in the presence of a polluting agent (60x)

A broad variety of stress conditions are able to mimic the effect of heat on gene expression, although with some species-specificity of inducers. It is possible that heavy metals and radionuclids from Uranium mines that reached Mureş Valley to act as inductors of large Balbiani ring-like structure at the right telomere of chromosome III, a phenomenon similar with heat shock response.

Conclusions

Based on our data, we suggest that the chromosomes of Chironomids can be used as testers of presence of genotoxic concentrations of polluting agents in aquatic ecosystems, because in the polytene chromosomes some biomarkers of environmental stressful conditions have been revealed: appearance of somatic rearrangements; changes in activity of *BR*s.

These somatic cytogenetic damages (changes) observed in cells of salivary glands of *Chironomus* larvae can provide an early warning signal of adverse long term effects in organisms including human beings.

One cannot exclude the probability that such damages can occur in germinal cells and therefore their presence in salivary gland cells is a signal of the extent of the risk to being transferred to the next generation.

Proving to be enough relevant at cytological level this polytene chromosomes system will be further used as genosensor for monitoring the environmental health status at ultrastructural and molecular levels.

Until now, we have identified most frecvent types of chromosomal aberrations and some structural and functional changes. These preliminary findings encouraged us enlarge the testing area, by increasing the number of the collected samples per region, and also the investigated regions exposed to pollution. Further, comparative investigation of natural populations is needed, including careful morphological analysis of all developmental stages, evaluation of the presence or absence of heterozygotes for any particular autosomal inversion or band polymorphism, and analysis of C banding. We also have to establish the frequency of the chromosomal aberrations.

Taken together, the results obtained in this preliminary study suggest that *Chironomus* sp. represents an efficient model system which can be used for environmental pollution monitoring under natural conditions.

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