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New aspects concerning the study of dipteran polytene chromosomal phenotype elements in *Chironomus plumosus*

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Abstract

*The cytogenetic analysis of dipteran insect **Chironomus plumosus** from Arad county (Mures valley) was performed looking for identification of some new polytene chromosomal phenotype elements which might be considered as genosensors in analysis of environmental pollution effects on the living systems. Chromosomal rearrangements like inversion or deletion loops identified in the polytene chromosomes were considered as potential genosensors appeared as a consequence of the environmental pollution. Other aspects of polytene chromosome phenotype like nucleolus-organizing function and aneuploidy as potential cytological elements with genosensitivity value have also been considered. C-banding staining of polytene chromosomes in **Chironomus plumosus** was performed for the first time in Romania while aneuploidy (monosomy and trisomy of the fourth polytene chromosome) is signaled for the first time in a Romanian **Chironomus plumosus** population.*

Keywords: polytene chromosomes, chromosomal rearrangements, genosensors, C-banding, puffing, nucleolus-organizer, aneuploidy.

Introduction

The study of polytene chromosome complement as an excellent genetic system for studying the chromosomal rearrangements and their evolutionary and adaptive values was inaugurated in the middle of the 20th century by Theodosius Dobzhansky[1], the greatest American Russian born evolutionist / geneticist.

Nowadays, the most widely polytene chromosomes phenotype elements used as genosensors are those of different *Drosophila* and *Chironomus* species [3][4][5][6].

Here we consider the informative potential as genosensors of different polytene chromosome phenotype elements of *Chironomus plumosus*, a typical aquatic organism, as larvae.

In previous papers[2][3][4] we analyzed the chromosomal rearrangements in *Chironomus* sp. as potential genosensors for monitoring environmental pollution.

We have studied the polytene chromosome complement of dipteran, taking advantage of the somatic synapses of such chromosomal structures in larval salivary – gland cells, that

provide a rapid image of the profound chromosomal rearrangements, because such chromosomal phenotype elements are easily identified and reflect both the molecular and cytological impairments of the genetic material with important consequences on the survival and the evolutionary perspectives of the species. Due to this environment protection involvement, the interest for such investigations is in an increased trend, all over the world.

The polytene chromosomes are very obvious interphase nuclear structures being in a somatic synapsis and as a consequence even some very small chromosomal rearrangements can be identified, a fact that with mitotic chromosomes is almost impossible in many instances even when modern molecular cytogenetic methods (i.e. chromosome banding or differential staining methods) are employed. In invertebrate group, the polytene chromosomes are found only in ciliates *Collembolae* and insects, but insects, especially dipteran species are quite ubiquitous in the nature so they can be easily found in different environments.

The bands and interbands patterns of the dipteran polytene chromosomes are highly reproducible and characteristic for a certain species. Any change in the structure of these chromosomes which creates a certain non-homology (through deletions, inversions and duplications, for example) can be seen at the level of the huge chromosome arms through the appearance of some alterations of the somatic pairing, expressed like loops. At the level of these loops, the polytene chromosomes can not pair any longer, showing therefore the duplicated state (paired, synapsed) of each chromosomal arm.

Material and methods

The chromosomes have been analyzed using an *Amplival* light microscope. Rapid staining method with 1% aceto-carmine was employed. Standard C-banding method using 5% barium hydroxide alcaline hidrolysis for 20 minutes was adapted with some modifications in our laboratory and Giemsa staining was also employed. The employment of an adapted G-banding method for *Ch. plumosus* polytene chromosomes is also in progress in our laboratory.

The *Chironomus plumosus* fourth instar larvae salivary glands were used. These were pulled out in a 1% aceto-carmine solution drop where they were kept for 15-30 minutes in order to obtain a proper staining. After that a coverslip on the material was set up and the squash method was used. The slides were visualized in light microscopy. Caution was taken in performing the squashing in order to avoid its possible secondary effects, such as false chromosomal aberrations (i.e. separations of synapsed chromatids or even fragmentation of the chromosomes).

Results and discussions

The polytenization process lacks the chromocenter formation in *Chironomus* species. Here the chromosomal arms remain as independent structures, each homologous chromosome making synapsis with its partner, and after that it suffers the polytenization process.

In *Chironomus plumosus* of Arad population, the individual polytene chromosomes could be identified according to their general length and were designated I-IV (fig. 1). Some extra polytene chromosomal structures with specific banding pattern, different from that of standard polytene chromosomes were identified in some 15% of the analyzed cells. We consider that these might represent B-chromosomes.

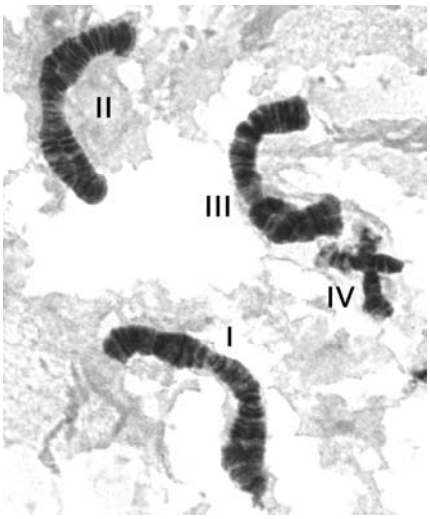

Figure 1 – The polytene chromosomal complement of *Chironomus plumosus*.

The trisomy of the fourth chromosomal pair, which contains nucleolus-organizer region was also encountered in the same cell with additional B-chromosomes (fig. 2). The future investigations involving the thorough identification of banding pattern specific for each chromosomal arm will clarify if these extra-chromosomal structures are derived from standard polytene chromosomes as a consequence of transposable elements activity as it was suggested by Siirin and coworkers (2003) [7] or they represent the centromeric fragment of the 4th chromosome as it was shown by their structure and pairing behaviour as Keyll and Hägele claimed (1971) [8]. Anyway the trisomy of the 4th pair is evident in our pictures, all these three polytene chromosomes being in an end-to-end synapsis at the level of the nucleolus-organizer region that exhibits decondensation. It means that this region is in an active state of transcription. The chromosomal polymorphism is a phenomenon often found in Dipterans, being a valuable tool for insect adaptation to different environmental conditions.

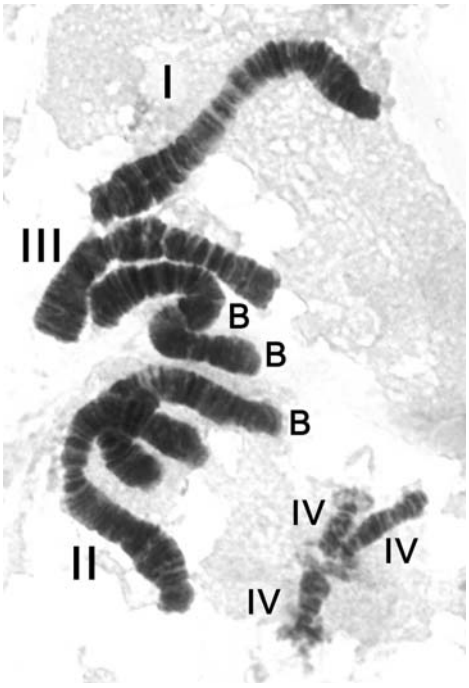

Figure 2 – Multiple additional B chromosomes in the polytene chromosomal complement (I-IV) of *Chironomus plumosus*. A trisomy of the nucleolar organizing chromosome (IV trisomy) can be observed.

The behaviour of the fourth pair of chromosomes - the nucleolus-organizing chromosomes- is very variable. In the majority of the cells these chromosomes pair in an end-to-end configuration (figures 2 - 5). In very rare instances they are paired in a side-by-side manner (fig. 6), while in most frequent instances they are completely separated and pulled apart at a long distance (figures 7 and 8). One can conclude that they were the object of extensive rearrangements all over their length that canceled their initial homology and this explains why these chromosomes when synapsed adopt the end-to-end manner.

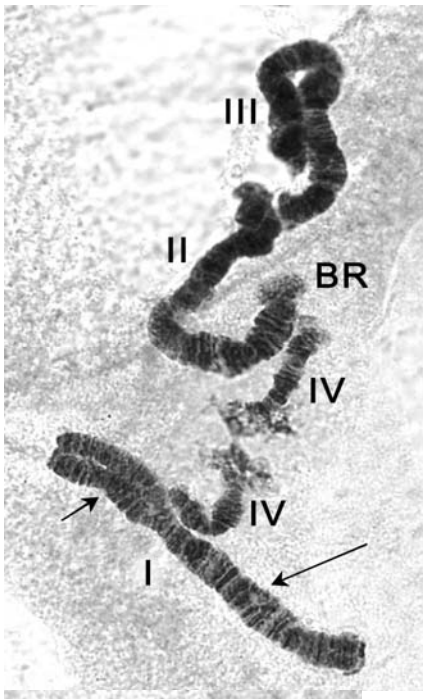

Figure 3 – The polytene chromosomal complement (I-IV) of *Chironomus plumosus* with a terminal inversion loop (short arrow) and an intercalar deletion loop (long arrow) in the chromosome I; a terminal Balbiani Ring in the chromosome II and the nucleolus-organizing chromosomes of the IV pair in an end-to-end synapsis at the level of their nucleolus-organizing region.

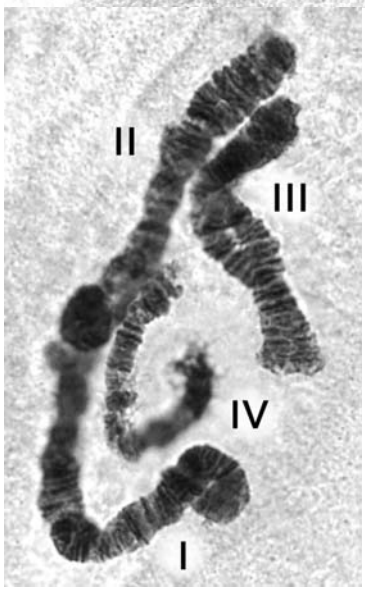

Figure 4 – Polytene chromosomal complement (I-IV) with a clear desynapsis loop resulting from a heterozygous intercalar deletion in the chromosome III and with the chromosomes of the IV pair in an end-to-end synapsis configuration.

A twisted terminal configuration giving rise to a thoroidal structure taking an eight figure appearing as two inversion loops was identified in chromosome I (see fig. 5). These first and third chromosomes are those that most often exhibit rearrangements.

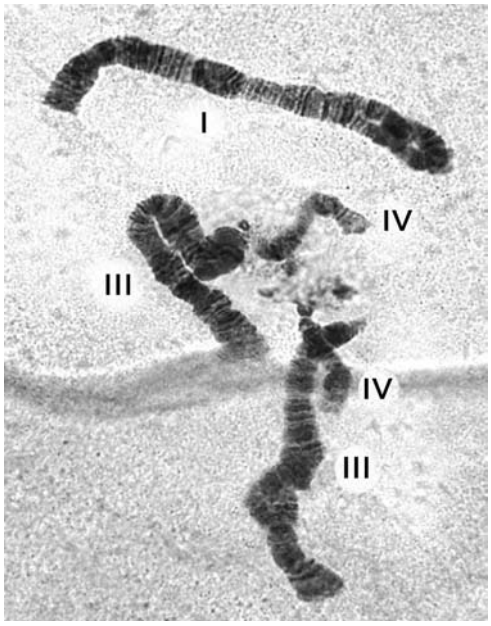

Figure 5 – Polytene chromosomal complement (I - IV) of ***Chironomus plumosus*** with a complex rearrangement appearing as a thoroidal terminal structure 8- shaped in chromosome I and terminal inversion loops in the chromosome III.

While in some cells (see figures 6 and 8) no chromosomal rearrangements could be identified, in other cells, multiple chromosomal structural aberrations could be recorded such as heterozygous terminal or intercalar inversions or deletions (figure 7).

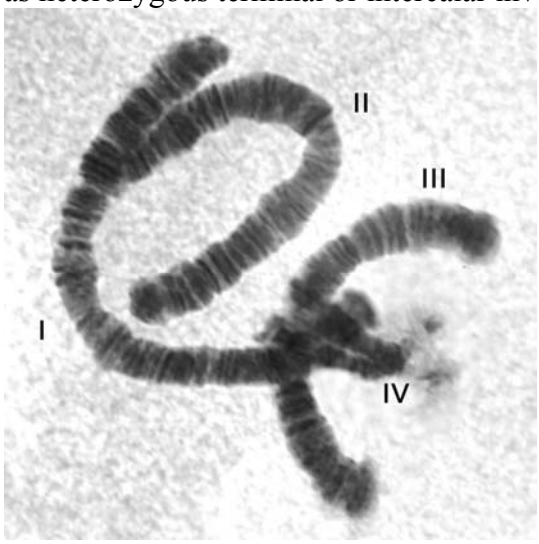

Figure 6 – Normal polytene chromosomal complement (I-IV) of ***Chironomus plumosus*** with the chromosomes of the IV pair partially synapsed in a side-by-side configuration at the level of the nucleolus-organizing region**.**

A possible monosomy of the fourth pair of polytene chromosome complement of *Chironomus plumosus* was occasionally encountered (fig. 9) in some cells of the same salivary gland in which the trisomy of this pair was also identified, suggesting that in the early embryonic development of this individual a mitotic nondisjunction event (i.e. failure of centromere division) in one of the two homologous chromosomes of the fourth chromosomal pair has taken place. This led to the appearance of two aneuploid cell-lines, one with trisomy (2n+1) (see figure 2), the other one with a monosomy (2n-1) (see figure 9) for this chromosomal pair. It means that the salivary gland of the larva was a mosaic of normal, disomic (2n=4), trisomic (2n+1=5) and monosomic (2n-1=3) cells if the polytene chromosomal complement is taken into account.

A twisted terminal configuration appearing as a thoroidal structure which is 8- shaped in the chromosome I, a heterozygous deletion loop intercalary located in chromosome II (long

arrow) and a terminal heterozygous deletion loop (short arrow) in chromosome III were identified.

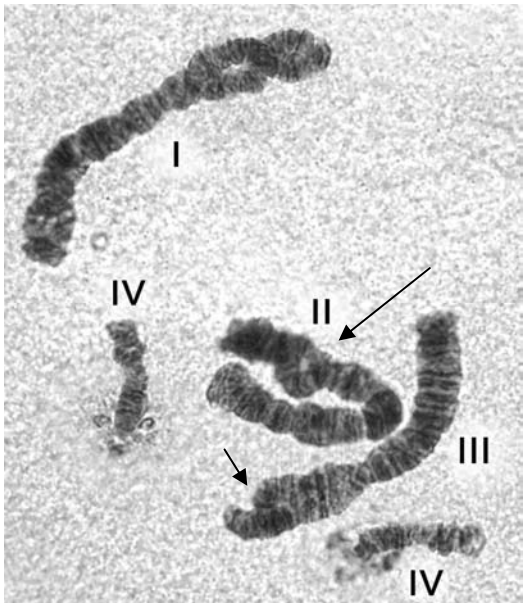

Figure 7 – Chromosomal rearrangements affecting polytene chromosomes I, II and III of the ***Chironomus plumosus*** chromosomal complement: double inversion loop in chromosome I; intercalar heterozygous deletion loop in chromosome II (long arrow) and terminal heterozygous deletion in chromosome III (short arrow).

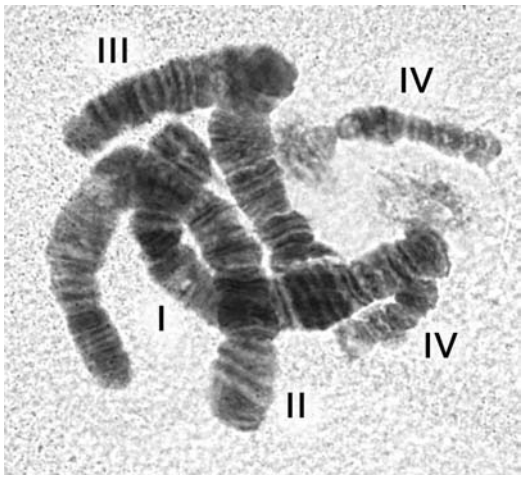

Figure 8 – Normal polytene chromosomal complement (I-IV) of *Chironomus plumosus* with the chromosomes IV completely separated**.**

In the same cell with monosomy of the fourth chromosomal pair a complex chromosomal rearrangement was identified in the polytene chromosome III, exhibiting the desynapsis of its short arm that appears as a fork with two branches holding between them a conspicuous, massive heterochromatic body that lacks a clear banding pattern. It might represent a translocation of an atypical B-chromosome. The desynapsis of the terminal region of long arm of the first and the third polytene chromosomes was also encountered. These chromosomal phenotype aspects might reflect the existence of some extensive disturbances in the somatic synaptic processes expressed at the level of the whole genome. The monosomy of the polytene chromosome IV is very well documented in some cells (fig. 9).

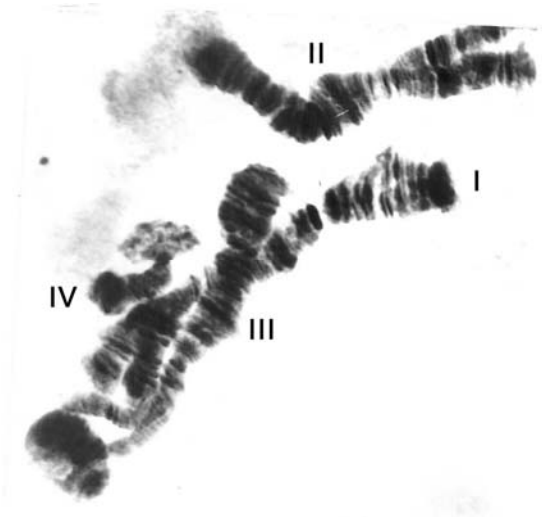

Figure 9 – The monosomy of the polytene chromosome IV in *Chironomus plumosus* in a cell with a translocated atypical B-chromosome between the branches of a desynapsis loop of the short arm of chromosome III.

A conspicuous terminal Balbiani ring of the chromosome IV represents its cytological landmarker, as it can also be seen both in monosomic (fig. 9) and in normal disomic for the fourth pair, cells (fig. 10). In figure 11 a desynapsis loop in the short arm of polytene chromosome III, an obvious intercalar desynapsis loop in the polytene chromosome II and an extensive decondensation of the short arm of the polytene chromosome I can be seen.

The monosomy of the polytene chromsome IV could be evidenced both in the cells with desynaptic loop of the short arm of chromosome III associated with a translocated atypical B-chromosome (fig. 9) and in the cells that contain only a desynaptic loop of this short arm of chromosome III without any association with other elements (fig. 11). On the other hand, the complex chromosomal rearrangement involving short arm of the polytene chromosome III and a translocated atypical B-chromosome was encountered both in monosomic for polytene chromosome IV cells (fig. 9) and in normal disomic cells for this chromosome IV (fig. 12). Therefore there is no doubt that this complex chromosomal rearrangement does not involve any interference of the polytene chromosomes III and IV, but merely polytene chromosome III and another chromosomal structure as we presumed to be an atypical B chromosome translocated between the branches of desynapsis loop of the short arm of polytene chromosome III (see figures 9 and 12).

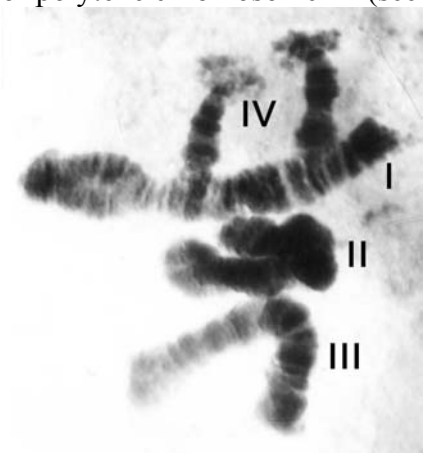

Figure 10 – The normal disomy for the fourth polytene chromosome in *Chironomus plumosus*.

A chromosomal rearrangement identical to that presented in figure 9 could also be encountered in the cells with normal disomy for the fourth polytene chromosome pair (fig. 12).

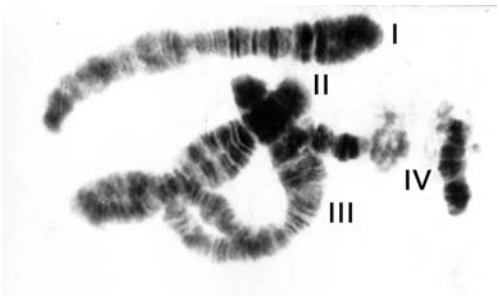

Figure 11 – *Chironomus plumosus* polytene chromosomal complement with an extensive decondensation of the short arm of chromosome I, an intercalar desynapsis loop in chromosome II and a desynapsis loop of the short arm of chromosome III that is not associated with an atypical translocated B-chromosome. Note polytene chromosomes IV are in an end – to –end synapsis.

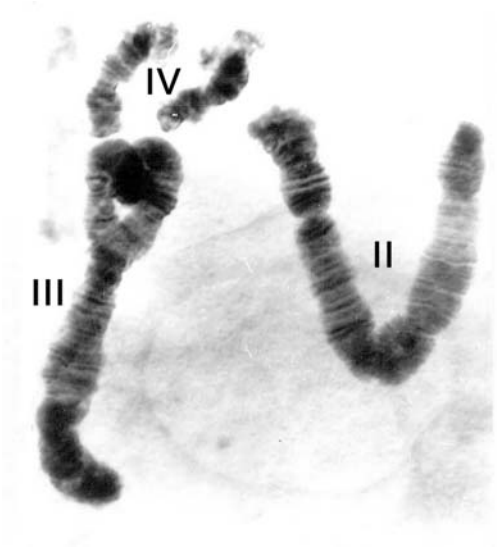

Figure 12 – Partial polytene chromosomal complement of *Chironomus plumosus* with a complex chromosomal rearrangement that involves the terminal desynapsis loop of the short arm of chromosome III associated with a presumptive translocated atypical B-chromosome, while the chromosomes IV and II are apparently normal. Note the disomy of the chromosome IV.

Employing C-banding staining method to *C. plumosus* polytene chromosomes, the constitutive heterochromatin of these chromosomes was evidenced (figures 13 and 14). It proved to be located in defined and very restricted areas on the polytene structures that are thinner than the remaining regions of the chromosomes. The constitutive heterochromatin was evidenced as conspicuous deep black transversal bands in the polytene chromosomes I and II (fig. 13) both of them exhibiting a desynapsis loop (arrows in figure 13) and an inversion loop (arrow in figure 14).

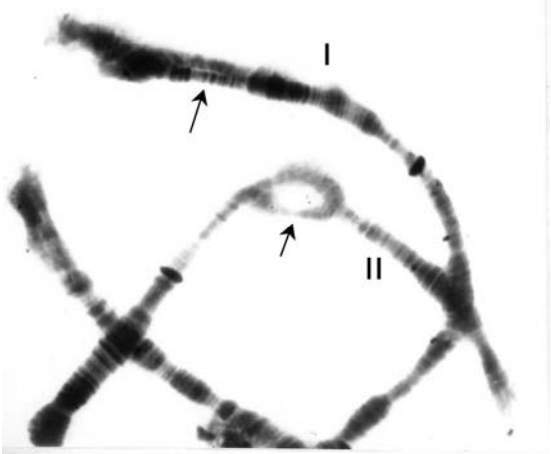

Figure 13 – C-banding of polytene chromosomes of *Chironomus plumosus.* The constitutive heterochromatin appears as large deep black stained bands in chromosomes I and II (arrows indicate desynapsis loops).

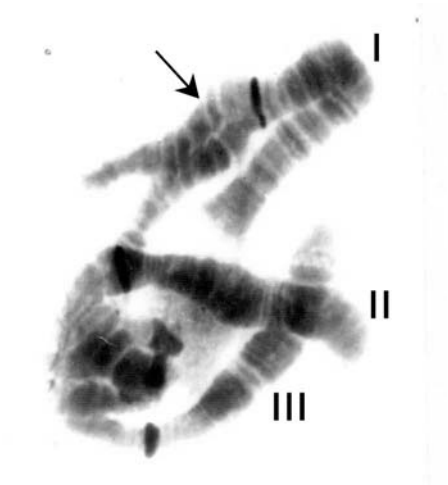

Figure 14 – C-banding of polytene chromosomes of *Chironomus plumosus*. The constitutive heterochromatin appears as long deep black stained transversal bands in chromosomes I and II and as a transversal black ovoidal band in chromosome III (arrow indicates a deletion loop).

Conclusions

Using fourth instar larvae of *Chironomus plumosus* in cytogenetic investigations looking to find some relevant genosensors useful to evaluate the health state of the environment, we could conclude that *Chironomus plumosus* can be a reliable model in studying the genosensors for the estimation of environmental pollution effects on living systems and genome dynamics of eukaryote cell. However, we consider that the chromosomal aberrations identified in different dipteran polytene chromosomes must be carefully investigated, caution being taken in considering some chromosomal rearrangements as being the direct effect of polluting agents. We must also take into account the interference of the genome of *Dipterae* with some genetic factors like transposable elements or genomic mutation represented by polyploidy or aneuploidy.

The careful examination of some polytene chromosomal phenotype elements encountered in the dipteran species collected from different polluted areas must be compared with the data from the literature or from previous original data obtained using dipteran species, collected from verified nonpolluted areas. A caution must be taken when such chromosomal rearrangements are used in monitoring the effects of environmental pollution on genetic material since, although they are very relevant and easy to be evidenced, some other factors could be at the origin of the genesis of some of such chromosomal structural abnormalities.

Our investigations revealed for the first time the existence of aneuploidy in an Romanian *Chironomus plumosus* population.

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