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ANALYSE OF THE POLYENE CHROMOSOMES IN OPTICAL MICROSCOPY, OF THE *CHIRONOMUS PLUMOSUS* SPECIES FROM THE POLLUTED AND CLEAN AREAS OF ARAD

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ABSTRACT: These conclusions are based on the results obtained in the study of the phenotype of polyten chromosomes with the identification of chromosomal rearrangements in normal conditions and pollution of the environment of polyten chromosomes from the larvae of one of the most important species of diptera: *Chironomus plumosus*. The number of these restructurations is far bigger and the restructurations much more complex at the recolted larvae from the zones considered critic under the aspect of pollution in the county of Arad, as opposed to those recolted from the zones considered unpolluted from the same county.Multiple types of chromosomal restructurations were evidenced, among which inversions, deletions, translocations, desinapsies, ectopic pairings, and also the existence od the aneuploids represented through monosomia and trisomia

Keywords: polytene chromosomes; *Chironomus plumosus*; pollution; chromosomes restructurations; inversions; deletions; translocations; monosomia and trisomia.

INTRODUCTION:

In Arad county, pollution represents an alteration process with more or less impact on the environment, it is created by some pollutants which come from different human activities such as household, agriculture, industry, transport, etc. The data obtained from the Agency for Environment Protection (2008 - 2016), shows us that the levels of pollution in Arad are normal, although there are some areas with high pollution risk and even critical levels. Considering the environmental elements which it affects, pollution can be: atmospheric, aquatic and of the soil.

The Dead Mures Canal is a deviation of the Muresel canal which flows in the Mures river, 30 km upstream of Arad. In the Dead Mures Canal is being evacuated polluted water, which comes from the industrial platform of the NV area of Arad, these industries including the mechanical industry, the food industry and the hotel industry. The polluted water is drained by the an purging station, which is under the Water Company of Arad jurisdiction. The station cleans the water only trough the mechanical step (sieving), which leads to the degradation of the quality of water. The Dead Mures Canal is considered to be in the V class of chemical environmental quality indicators (due to RO, Nutrients, Salinity), in the IV of biological frame and in the IV of the ecological one, on all the 20 km radius.

On the Mures Valley, in the areas of Milova, Odvos and Barzava, where uranium deposits and mines exist, the vegetation, water and soil samples show higher than normal levels of pollution. A special area is the small city of Ranusa, situated on the right side of the Cretului Valley, where during a period of 30 years there have been exploitations until 1993, when the conclusion that the deposits don't have a high quality and their exploitation isn't economically beneficial was reached. From this activity has resulted a tailing with a volume of 256.000 cubic meters, situated on a 1 km area.

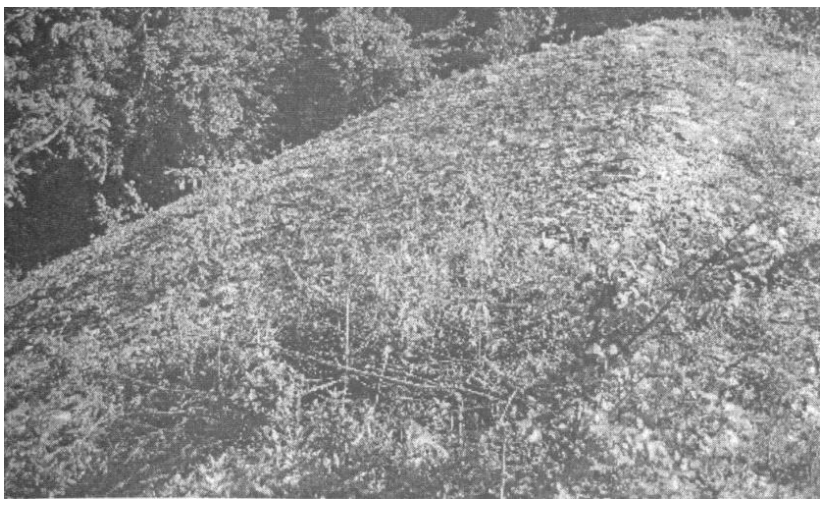

Fig. 1. The Ranusa Tailing

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POLYTENE CHROMOSOMES (GIANT OR HUGE)

Polytene chromosomes begin as normal chromosomes from somatic cells, which divide mitotically up to a point, whereupon they enter an endoreduplication cycle, replicating their genetic material more times (Dobzhanski *et al.*, 1977). But, after every DNA replication round, chromatids separation in Y chromosomes stops taking place. The division of the nucleus also stops: the numerous rounds of synthesis of chromatic material take place inside of the one and only nucleus, which thereby becomes a giant, huge nucleus (Petrova, 1991).

Through endoreduplication, a large number of chromatides appears, which remains in a parallel disposition, which leads to the occurence of a polytene structure, resulting in a giant chromosome(Gavrila (1983). Gavrilă and the contributors (2003, 2008, 2009), see the polytene chromosomes as excellent working structures, which guarantee the synthesis of some necessary products in a very short period of time and in a large quantity, which conditions the transformation of the larva in stern. After the synthesis of these products takes place, the cells producing this kind of synthesis suffer from the autolysis process (Lima de Faria, 1983) .

MATERIALS AND METHODS:

1. Harvested species

The analyzed biological material was harvested from different areas in Arad County (Rănușa, Arad and Mureș River Valley) more or less polluted by different substances and is represented by the larvae of the *Chironomus plumosus* species, which belong to the Diptera order, Insecta class.

Fig. 2. *Chironomus plumosus* larva collected from Dead Mureș River Channel, Arad

2. Working methods

The methods regarding highlighting the polytene chromosomes are: the quick method implying coloring with carmine-acetic, the Feulgen method.

THE FEULGEN METHOD

Materials

Stage III-IV larvae of *Chrionomus plumosus*

Fixative (absolute methylic alcohol : glacial acetic acid 3:1)

HCl 1N

Schiff Reagent (Basic Fuchsine decolorized with Potassium Metabisulfite)

Acetic carmine

Coloring Technique Steps

a. Samples Collection

- Stage III-IV larvae are used

Salivary Glands are located in the anterior part of the body, behind the mouthpiece, which looks like a black dot. The larva is watched at Binocular magnifying glass. The tip of a needle is placed behind mouthpiece and the tearing operation in a longitudinal direction reported to larva body is carried out. Together with the mouthpiece the salivary glands will be extracted.

b. The fixation

The fixation takes one hour in absolute methyl alcohol: glacial acetic acid 3:1 in the refrigerator (40˚C).

The cells are being killed and the cytoplasmic content is coagulated while the nuclear structures are preserved.

c. Hydrolysis

Hydrolysis is done with HCl 1N at 60˚C for 4 minutes. By hydrolysis the coloring process and afterward the display of the cells on a blade is eased

d. Coloring

The biological material extracted from HCl 1N is introduced into distilled water. The salivary glands are then transferred to a piece of filter paper for dehydration and then inserted into the dye.

The material is left in the Schiff reagent overnight in the refrigerator.

e. Preparations

The material is placed at microscopic blade and few drops of acetic-carmine are added in order to intensify the coloring process. The preparation is done by applying a blade over the drop of fresh carmine or acetic water (45% glacial acetic acid, 55% distilled water) In which the salivary glands are located. Then, it is gently pressed with the spatula needle and after

that with the filter paper in order to absorb the liquid excess.

The top of a match stick can be used for displaying. With it lightly knocking can be done for a squash that holds the band pattern.

After drying in the air, the preparation is analyzed with the microscope with 40 lens or immersion lens.

RESULTS AND DISCUSSION:

The chromosomal set in *Chironomus plumosus* is 2n=8 and the chromosome arm combination is AB, CD, EF and G. AB(I) and CD(II) chromosomes are metacentrics and are the largest , while EF(III) is submetacentric and G(IV) chromosome is telocentric. The first three pairs of chromosome from *Chironomus plumosus* represents 90% of this species genome(Michailova *et al.*, 1991, 2009). Figure No.3 shows the chromosome polythene complement in *Chironomus plumosus* with those four assigned polythene chromosomes I-IV, each of which representing somatic bivalents. The polythene chromosome IV fulfills the function of a nucleotide organizer presenting a terminal sector where the cromomers are hypercondensed and a diametrically opposed subterminal sector where the cromomers is in a maximum stadium of despiralization, here being localized the organizing nucleolar region which shelters the ribozomal cistrons.

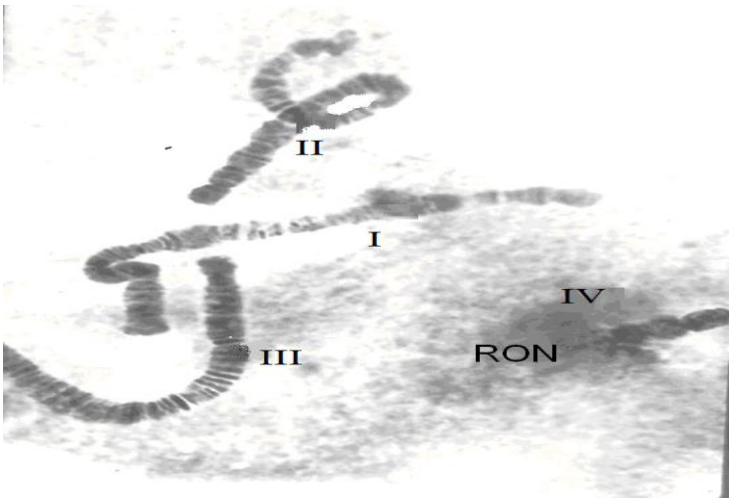

Fig. 3. The chromosomal complement at *Chironomus plumosus* from Arad County

At *Chironomus plumosus* during the process of politenization there is not a formation phase of the chromocentre, the chromosomial arms are isolated and independent, every chromosome making a sinapse with his counterpart, after that they suffer the process of politenization.

The Balbiani rings are permanently active puffers with genes that encode the principal polipeptides of the salivary glands. Generally, in an unpopulated ecosystem, the three Balbiani rings are almost identycal in shape. In comparisson, among the studied population, there was noticed a dramatic reduction of the BRc, simultaneously with the expansion Bra(No) (Carabas, 2010).

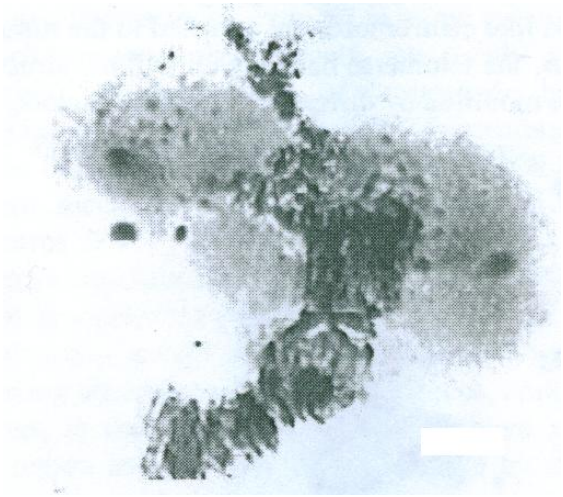

Fig. 4. The expansion of the BRa(No) from the fourth chromosome, which functions as RON and highlights the duplicated state at the *Chironomus plumosus*.

Through the DAPI coloration a remarkable telomeric puffer was highlighted for the population of *Chironomus plumosus* collected from Rănușa. It is possible that the hard metals and radionuclides from the uranium mines which polluted those areas to act as inductors of the Balbiani ring massive structure at the telomer from the right side of the third chromosome, a similar phenomenon to the response to thermical shock.

Any modification in the structure of politen chromosomes at the *Chironomus* leads to the appearance of some alterations at the somatic pairings expressed as curls, on which can be noticed the duplicated state of the chromosomal arms which represents an excellent system of studium of chromosomial rearrangements which is formed in geno-detectors for the appreciation of the health of the surrounding environment and the highlight of the deadly effects of her alteration due to pullution or the intervention of some abnormal meteorological conditions (thermical shocks).

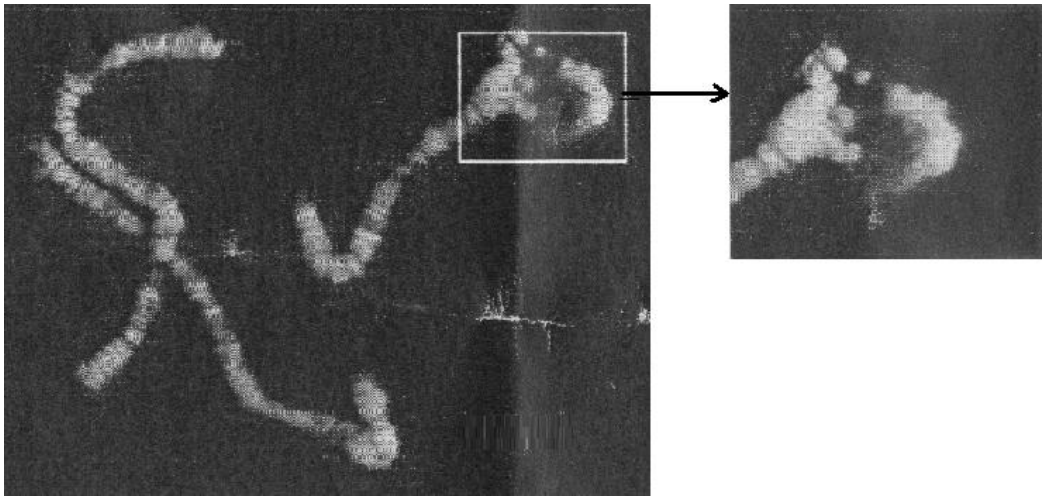

Fig. 5. The telomere puffer of chromosome III of *Chironomus plumosus* shows a possible activation of the HS type genes as a response to the stress from the outer environment caused by the presence of a pollutant agent.

In Figure 6 different spirals can be observed: determined by either inversion, deletion, or desynapse, as well as a telomere to telomere synapse configuration at the chromosomes from the IV pair (Carabas, 2010).

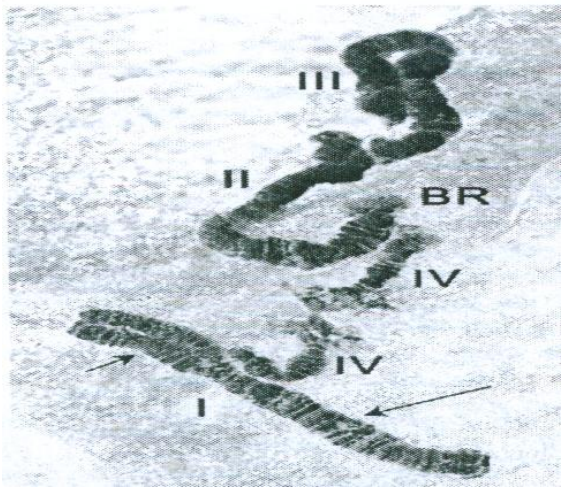

Fig. 6. Politen chromosomal complement (I-IV) of *Chironomus plumosus,* with a terminal inversion curl (the short arrow) and an interleaved deletion curl in chromosome I; one Balbiani terminal ring in chromosome II and the nucleolar organizer chromosomes from pair IV situated in a telomere to telomere synapse at the nucleolar organizing region.

In the politen chromosomal complement (I-IV) of *Chironomus plumosus* can be observed extensive chromosomal rearrangements (Figure. 7): desynapse of the long arm of the I chromosome with a union at the telomeres of the separate arms which provides it the appearance of the number eight. This chromosome is associated with a chromatic fragment (FC) ectopically geminated at the level of a desynapse or inversion curl in the short arm of the chromosome I, chromosome from the IV pair and the long arm of its' homologous associated with a translocation of an acentric fragment. A deletion/inversion curl in vicinity of the centromer of the chromosome III with desynapse at the level of the short arm telomeres of this chromosome with an

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interleaved curl of inversion/deletion in one of the arms of the chromosome II and a possible telomere to telomere translocation between chromosomes II and III.

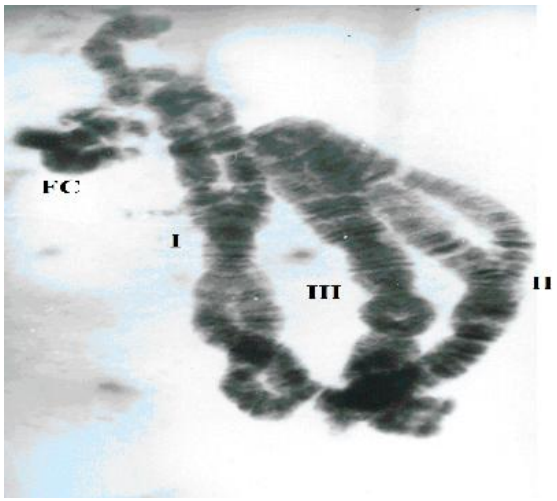


Fig. 7. Ample chromosomal rearrangements in the polythen chromosomal complement *Chironomus plumosus.*

Desynapse of the long arm of the first chromosome with the union at the level of telomers of the separate arms which offers him the aspect of the eight number.

Besides the chromosomal restructuring, in the studied population of *Chironomus plumosus* in Arad county, were identified genomic mutations represented by aneuploidies (monosomy, trisomy) which affects the IVth pair of chromosomes and also the numerical variation between 0 and 3 presentation presence of the supernumerary chromosomes, designated as B chromosomes. It is known that the nature of B chromosomes is heterochromatic. They have an important role, affecting the genomes plasticity and offering adaptive elasticity, to the variations of environmental conditions , to the wearer.

In Figure.8 it is represented a clear case of monosomy for the IVth pair, this one being represented in the chromosomal complement just in one copy. The monosomic condition IV is associated with numerous structural chromosomal reshaping. So, in the I st chromosome appears a loop of desinapsis in the long arm, between its two branches, a stong heterochromatised chromosomal fragment being translocated. I st chromosome's short arm has a discreet loom of deletion with the elimination of only one dark band. The II nd chromosome has a terminal desinapsic loop in the long arm and the change of the banding pattern in the short arm. The III rd chromosome has adesinapsic loop at the long arm's level associated with a terminal deletion in one of its separated branches. The IV th chromosome appears normally structured, having a sextensive puffer RON.

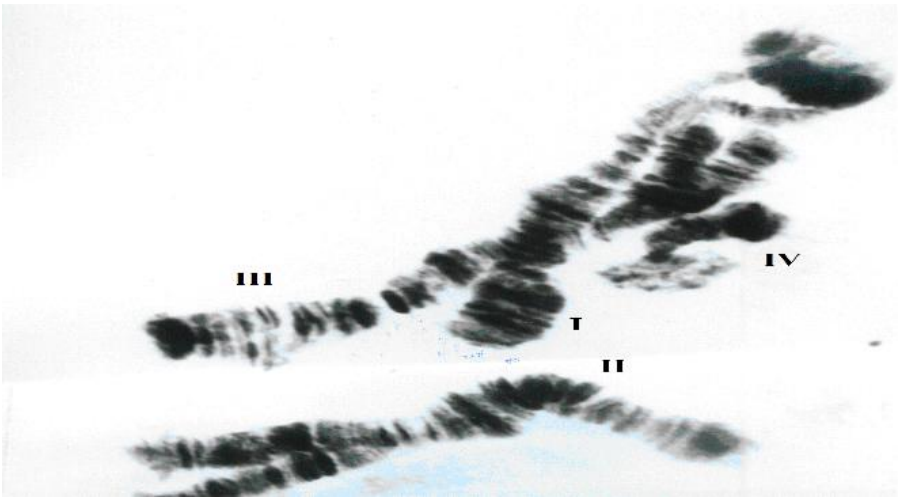


Fig. 8. Chromosomal politen complement to *Chironomus plumosus* with monosomy for the IV th pair, associated with numerous structural chromosomal reshaping in other chromosomes.

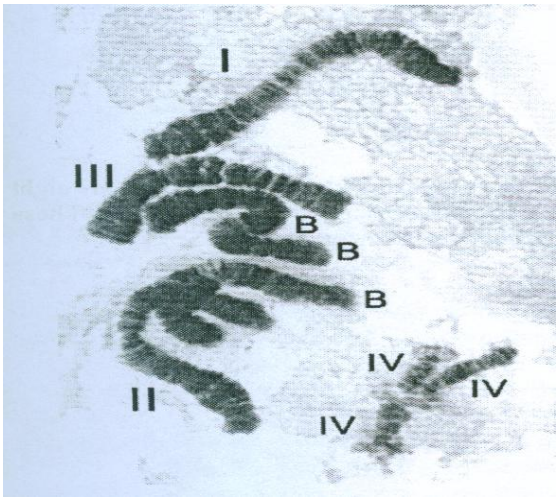
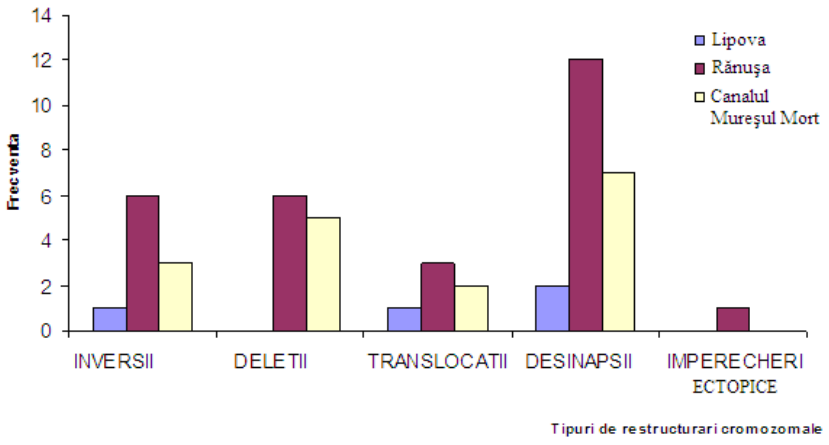


Fig. 9. Multiple chromosomes B in the polymorphic chromosome complement (I-IV) of *Chironomus plumosus*. There is a trisomy of the nucleolar organizer chromosome (trisomy IV).

A total of 100 chromosomal complements were analyzed for 35 individuals for each of the three populations of two polluted areas, represented by the Mort Mureș Channel in Arad and Rănușa and a population from an unpolluted area located in the Lipova locality.

Tab. 1.
Frequency of structural and functional somatic aberrations in *Chironomus plumosus* in polluted areas and control area.

AREA	**No individuals analyzed (100)**	**No polythene nuclei (100)**	**Spectrum of chromosomal rearrangements (no / 100 polythene nuclei)** INVERSII	DELETII	TRANSLOCATII	DESINAPSII	IMPERECHERI ECTOPICE
CONTROL (CLEAN) LIPOVA	35	100	1	0	1	2	0
POPULATION 1 (RADIOACTIVE) RĂNUȘA	35	100	6	6	3	12	1
POPULATION 2 (HEAVY METALS) MUREȘUL MORT ARAD	35	100	3	5	2	7	0



Graph. 1. The estimation (histogram) of the chromosomal rearrangements for *Chironomus plumosus* specie in the investigated areas in Lipova, Ranușa and Dead Mureș Channel (Canalul Mureșul Mort)

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Between the control zone and the polluted zones, but also between the polluted zones, there are differences regarding the chromosomal modifications.These differences can be explained through numerous factors, including the different concentrations of heavy metals and their compounds, the intensity(dose) of radiation, and on the other hand the specific differences of life conditions for each studied biotope, like the physiological stage or the organism in which the polluting agent acted.

CONCLUSIONS:

These conclusions are based on the results obtained in the study of the phenotype of polyten chromosomes with the identification of chromosomal rearrangements in normal conditions and pollution of the environment of polyten chromosomes from the larvae of one of the most important species of diptera: *Chironomus plumosus.*

The number of these restructurations is far bigger and the restructurations much more complex at the recolted larvae from the zones considered critic under the aspect of pollution in the county of Arad, as opposed to those recolted from the zones considered unpolluted from the same county.

Multiple types of chromosomal restructurations were evidenced, among which inversions, deletions, translocations, desinapsies, ectopic pairings, and also the existence od the aneuploids represented through monosomia and trisomia.

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