PROCEEDINGS OF THE BALKAN SCIENTIFIC CONFERENCE OF BIOLOGY IN PLOVDIV (BULGARIA) FROM 19TH TILL 21ST OF MAY 2005 (EDS B. GRUEV, M. NIKOLOVA AND A. DONEV), 2005 (P. 744–752)

THE EFFECT OF LEAD ON THE POLYTENE CHROMOSOMES OF *CHIRONOMUS PIGER* STRENZKE (DIPTERA, CHIRONOMIDAE)

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ABSTRACT. The chromosome variability of *C. piger* (Diptera, Chironomidae) is studied after treatment with lead nitrate. The cytogenetic analysis revealed some structural and functional changes in polytene chromosomes. The structural aberrations – heterozygous inversions, deletions and deficiency, are somatic and occurred very rarely. Their values are not significantly different compared to the control. The genome of the phylogenetically older *C. piger* reacts to stress factor mainly by alterations in the functional activity of chromosomes. In contrast, many structural aberrations with high frequency are found in the phyligenetically younger species *C. riparius* after lead treatment. It was confirmed that the chromosome changes are species – specific and depend on the structural organization of the genome. These changes could be used as a tool for assessment of genotoxicity in aquatic ecosystems. It was hypothesized that *C. piger* genome is more stable to stress compared to some other chironomid species.

KEY WORDS. Chironomidae, toxic effect of lead, chromosome alterations

INTRODUCTION

Many studies have shown that lead ions have harmful and toxic effects on different organisms. Pb ions damage chromosome structure and induce an increase in chromosome aberrations in mammalian species (Paton, 1973; Tachi & Nashime, 1975; Dhir et al. 1990; Hartwig et al. 1990). Some chromosome changes - aberrations, destroyed conjugation, are found in Diptera species from genus *Anopheles* and *Drosophila* (Sharma et al. 1988; Winder & Bonin, 1993). Experiments show that Pb causes different alterations in polytene chromosome structure and function in model chironomid species (Michailova & Belcheva, 1990; Michailova et al. 2002; Belonogova & Belyanina, 2001). It was found a dose-dependent association

between the incidence of active puffs and lead concentrations (Michailova et al. 2002), along with a lot of somatic structural aberrations (Michailova et al. 2001b). These authors discuss the idea that genotoxicity of lead and the morphofunctional characteristics of chromosomes could serve as a criterion for toxicity of various stress factors in biotopes.

The aim of this study is to establish the sensitivity of *C. piger* genome to lead ions influence.

MATERIAL AND METHODS

The experiment was carried out in laboratory conditions (Michailova, 1985): photoperiod 16h light and 8h dark, 18-20^{o}C temperature and constant aeration. The fertilized egg mass was exposed to 151 mg/l Pb(NO3)2 stock solution (Merck, Germany). The control was reared in dechlorinated water.

Four instar larvae were fixed in alcohol:acetic acid (3:1). Permanent polytene chromosome preparations were made according to the aceto-orcein method (Michailova, 1989).

Standard chromosome maps of *C. piger* (Hagele, 1970; Kiknadze et al. 1991) were used for species differentiation and cytotagenetical analysis. The number of studied cells and specimens are shown in table 1. The frequency of the chromosome aberrations is calculated as a percentage of the total number of cells. The functional activity of Nucleolar organizer (NOR) and Balbiani rings (BRs) was measured by the degree of puffing: high activity (++), intermediate or low (+) and no activity (-) (Beermann, 1971). The results concerning the levels of NOR and BRs activity were analyzed by Student's t-test ($P<0,05$). The results of decondenzation and asynapsis were analyzed by G-test.

Chemical analysis of larvae tissue was made for detecting accumulation of lead ions in the body after treatment.

RESULTS

1.1. Cytotaxonomical characteristics of control polytene chromosomes:

C. piger Srtenzke belongs to „thummi" complex with a chromosome arm combination AB (I), CD (II), EF (III), G (IV). It has a chromosome set 2n=8 and good band chromosomes structure. In chromosome G are localized three Balbiani rings (BRa, BRb, BRc) and a Nucleolar organizer (NOR).

The individuals from the control did not differ cytogenetically from the standard karyotype (Keyl & Strenzke, 1956; Keyl, 1962, Kiknadze et al. 1991). After analysis some structural alterations were found but with a very low frequency – pericentric heterozygous inversions in AB centromere regions (2 %) and a heterozygous inversion in arm C (B3-B5), which affected one cell only. Deletions of BRc in chromosome G were detected (1,07%). Besides the structural aberrations, changes in chromosome function were established more frequently. Arms A, D, F and G participated in ectopic contacts very rare (0,33 - 0,67 %). Most often we found asynapsis in arm G (47,33 %) and in AB centromere region (14,33 %). Their frequencies are significant compared to the treated material ($P<0,05$). Asynapsis in

arms A, B, C, F and EF centromere region also occurred with a very low frequency. It was found also decondenzation of telomere regions in all chromosomes. Highest frequency of telomere decondenzation was detected in arm D (22,67 %), arm C (18,33 %) and arm A (11,33 %).
In respect to BRs and NOR activity, different states and changes in their normal activity were observed. Most often BRc/BRb occurred in +/- state (46,24 %), followed by ++/- (21,9 %) and -/- (19, 42%) (Fig.1a). The state of low activity of NOR (+/+) prevailed (60,08 %) but NOR also appeared in high activity. Cases of not active NOR were also registered (Fig. 1b).

1.2. Cytotaxonomical characteristics of polytene chromosomes of treated material:
The cytogenetic analysis of polytene chromosomes after treatment showed some changes in chromosome structure and function. The structural aberrations found are somatic heterozygous inversion in arm E, deficiency in G chromosome, deletions of bands in section A of G chromosome and deletions of either BRc or both BRc and BRb. The deletions of BRc occurred in heterozygous state (1,24 %) as well as in homozygous state (2,48 %). As a result of BRc/b deletions we found an active chromosome G, so called "pompon" -like chromosome but in one cell only. Statistical analysis of structural aberrations did not show any significant differences between treated material and control.
The changes in chromosome function are more frequently. They included decondenzation of telomere regions, asynapsis, ectopic contacts and changes of BRs and NOR activity. All chromosomes participated in ectopic contacts (0,5 % - 1,73 %). Most often asynapsis affected chromosome G (20,05 %) and arm E (11,14 %). The values of decondenzation of arm D (32,18 %), arm A (27,72 %) and arm C (12,62 %) were the highest. The frequency of arm A decondenzation differs significantly compared to the control ($G=8,65$, $P<0,01$).
Different levels of BRs and NOR activity were observed. Most frequently (49,93 %) occurred +/- state of BRc/BRb when BRc is low active and BRb has no activity. It was significantly different compared to the other states in treated material ($P<0.05$) (Fig. 1a). BRc/BRb were observed also in completely suppressed state -/- (22 %).
The high activity of NOR was suppressed and more frequently it appeared in low active state +/+ (67,41 %) but it was observed also in heterozygous state and without any activity (Fig. 1b). The level of high activity occurred only in 15,7 %. The state of low activity is significantly higher compared to the other six states (Fig. 1b). The heterozygous state +/- showed significant difference between the control and the treated material ($P<0.05$).
It was not found any accumulation of Pb ions in larvae tissue (0,05 mg/g).

DISCUSSION

The results of our experiments show that Pb influences mostly the functional activity of *C. piger* genome. Decondezation and asynapsis in all chromosomes were found. However, the differences in the frequencies of these changes between the control and the treated material were almost minimal. Significantly higher compared to control

are the frequencies of low activity of BRc/BRb and the heterozygous state of NOR as well as the value of the decondenzation of arm A telomere. Despite of the observed changes in control, the total value of the normal high activity of BRc is higher (26,93 %) compared to the treated material (12,71 %). Similar is the case of high activity of NOR in the control.

The structures like BRs and NOR are very sensitive to stress factors (Diez et al. 1990; Michailova et al. 1998). In treated material the high activity of BRs and NOR was suppressed and the frequencies of states +/- of BRc/BRb and +/+ of NOR are significantly higher compared to the other levels. Suppression of high activity of Balbiani rings and Nucleolar organizer is found in some chironomid species after stress in laboratory experiments and as well as in natural populations living in anthropogenically polluted regions (Aziz et al. 1991; Todorova et al. 2000; Michailova et al. 2001; Michailova et al. 2003). The reduction of their high activity is a result of the production of heat shock proteins, which suppress RNA synthesis (Aziz et al. 1991). These proteins support survival and adaptation of the individuals in extreme conditions (Michailova, 2002). Increasing of metalothionein proteins synthesis is possible too. These proteins are able to bind with the metals in the tissue and could detoxicate their effects (Roesijadi, 1992). This may explain the lack of Pb ions accumulation in *C. piger* tissue (0,05 mg/g). The Chironomids are very tolerant to heavy metals (Wetsel et al. 1978) and a mechanism for regulation and excretion of the metal from the body may exist.

The frequency of the observed deletions of BRs after treatment is higher (7,44 %) than in the control (1,07 %). The lead ions cause appearance of a "pompon"-like chromosome G, as described as Michailova et al. (1996). "Pompon" is found in *C. riparius* as a result of Pb and Cr exposures (Michailova et al. 2001a,b) and in natural population in anthropogenic polluted regions (Michailova et al. 1996: Todorova, 2000) and could serve as a cytogenetic biomarker for heavy metal pollution (Michailova et al. 1998).

The chromosome G is the most unstable in regard to various impacts but in *C. piger* it is not so well expressed as is in the phylogenetically younger species *C. riparius*. In *C. riparius* after lead exposure many somatic structural aberrations in the chromosomes along with functional changes are found (Michailova et al. 2001b).

Similar results are observed in other sibling species – *Gl. salinus* and *Gl. barbipes* (Michailova et al. 2002; Michailova, Belcheva, 1990). *Gl. barbipes* is phylogenetically younger and reacts to lead influence through different structural aberrations (Michailova, Belcheva, 1990). In contrast, in *Gl. salinus*, which is older, various functional changes in the chromosomes are detected mostly (Michailova et al. 2002). The phylogenetically old species *Gl. pallens* mobilizes its genome to stress mostly by functional alterations (Todorova et al. 2000). The chromosome changes concerning the function or both chromosome structure and function are species-specific reactions depending on the structural organization of the genome (Michailova et al. 2002).

The significant differences in the observed changes between the control and treated material are almost absent, so we could conclude that *C. piger* genome is more stable

to such an influence. On the other hand, functional changes are found and we could say that the chromosomes of the phylogenetically older species are more sensitive to stress influence concerning their functional activity. However, more precise data necessitate more experiments to be performed with this species.

ACKNOWLEDGMENTS

This study was performed in lab "Cytogenetic and evolution" and granted by Royal Society. The chemical analysis of larvae tissue has been done by Dr K. White from the University of Manchester. I would like to thank prof. P. Michailova from the Institute of Zoology- BAS for her competent advises.

REFERENCE

AZIZ, J. B., AKRAWI, N. M., NASSORI, G. A. 1991. The effect of cronic toxicity of copper an the activity of Balbiani rings and nucleolar organizing region in the salivary gland chromosomes of *Chironomus ninevah* larvae. Enviro. Pollut., 69 (2-3): 125-130.

BEERMAN, W. 1971. Effect of L-amanitine of puffing and intranuclear RNA synthesis in Chironomus salivary glands. Chromosoma, 34, 152-164.

BELONOGOVA, BELJANINA, 2001. Changes in genetic activity of Chironomidae polytene chromosomes after lead influence. In "Small rivers: Recent ecological statement and problems", Intern. Sci. Conf. Toliatti, p.45 (in Russian).

DHIR, H., KUMAR, A., SHARMA, A., TALUKDER, G. 1990. Modification of clastogenicity of lead and aluminium in mouse bone marrow cells by dietary ingestion of *Phyllanthus emblica* fruit extract. Mutation research, 241, 305-312.

DIEZ, L., CORTES, E., MERIMO, J., SANTA CRUZ, M. 1990. Galactose induced puffing in *Chironomus thummi:* Balbiani rings and their significance in protein synthesis. Chormosoma, 99, 61-70.

HARTWIG, A., SCHLEPEGRELL, R., BEYERSMANN, D. 1990. Indirect mechanism of lead-induced genotoxicity in cultured mammalian cells. Mutation research, 241, 75-82.

HAGELE, K. 1970. DNA – Replikationsmuster der Speisheldrusen chromosomen von Chironomiden. Chromosoma, 33, 297-318.

KEYL, H. 1962. Chromosomenevolution bei Chironomus. II. Chromosomenumbauten und phylogenetische Beziehungen der Arten. Chromosoma, 13, 496-541.

KEYL, H., STRENZKE, K. 1956. Taxonomie und Cytologie von zwei Subspecies der Art Chironomus thummi. Zeitschr. Naturforsch, 116, 727-735.

KIKNADZE, I.I., SHILOVA, A., KERKIS, I., SHOBANOV, N., ZELENTZOV, N., GREBENCHJUK, F., ISTOMINA, A., PRASLOV, B. 1991. Karyotype and morphology of larvae in Chironomini. Atlas, Novosibirsk. (in Russian).

MICHAILOVA, P. 1985. Method of breeding the species from family Chironomidae, Diptera in experimental conditions. C.R. Acad. Bulg. Sci. 9, 1179-1181.

MICHAILOVA, P. 1989. The polytene chromosomes and their significance to the systematics of the family Chironomidae, Diptera. Acta Zool. Fennica, 186, 1-107.

MICHAILOVA, P. 2002. The effect of anthropogenic factors on polytene chromosome structure and function in insects of family Chironomidae, Diptera. Oncologus, 2, 43-47 (in Bulgarian).

MICHAILOVA, P., BELCHEVA, P. 1990. Different effects of lead on external morphology and polytene chromosomes of *Glyptotendipes barbipes* (Staeger), Diptera, Chironomidae. Folia Biol., 38: 83-88.

MICHAILOVA, P., PETROVA, N., RAMELLA, L., SELLA, G., TODOROVA, J., ZELANO, V. 1996. Cytogenetic characteristics of a population of *Chironomus riparius* Meigen 1804 (Diptera, Chironomidae) from a polluted Po river station. Genetica, 98: 168-178.

MICHAILOVA, P., PETROVA, N., SELLA, G., RAMELLA, L., BOVERO, S. 1998. Structural-functional rearrangements in chromosome G in *Chironomus riparius* (Diptera, Chironomidae) collected from a heavy metal – polluted area near Turin, Italy. Environmental Pollution, 103: 127-134

MICHAILOVA, P., PETROVA, N., SELLA, G., BOVERO, S., RAMELLA, L., REGOLI, F, ZELANO, V. 2001a. Genotoxic effects of chromium on polytene chromosomes of *Chironomus riparius* Meigen 1804 (Diptera, Chironomidae). Carylogia, 54 (1): 59-71.

MICHAILOVA, P., ILKOVA, J., PETROVA, N., WHITE, K. 2001B. Rearrangements in the salivary gland chromosomes of *Chironomus riparius* Mg. (Diptera, Chironomidae) following exposure to lead. Caryologia, 54, 4, 349-363.

MICHAILOVA, P., TODOROVA, K., WHITE, K. 2002. The effect of lead on the salivary gland chromosomes of *Glyptotendipes salinus* Michailova (Chironomidae, Diptera). Biologia, Bratislava, 57 (3): 359-367.

MICHAILOVA, P., ILKOVA, J., WHITE, K. 2003. Functional and structural rearrangements of salivary gland polytene chromosomes of *Chironomus riparius* Mg. (Diptera, Chironomidae) in response to freshly neutralized aluminium. Environmental Pollution, 123, 193-207.

PATON, G. 1973. Effects of certain metal ions on DNA repair in mammalian cells. Mut. Res., 21, 199-201

ROESIJADI, G. 1992. Metallothioneins in metal regulation and toxicity in aquatic animals. Aquatic Toxicology, 22: 81-114.

SHARMA, G. P., SOBTI, R. C., CHAUDHRY, A., AHLUWALIA, K. K. 1988. Genotoxicity of two heavy metal compounds – lead acetate and mercuric chloride in the mosquito *Anopheles stephensi* Liston (Culicidae: Diptera). Cytologia, 53, 263-267.

TACHI, K., NISHIME, S. 1975. Cytogenetic effects of lead acetate on rat bone marrow cells. Arch. Environ. Health, 403, 144-147.

TODOROVA, J. 2000. Cytotaxonomic variability of *Chironomus riparius* Meigen (Diptera, Chironomidae) from anthropogenically influenced regions in Bulgaria. Acta Zool. Bulg., 52 (2): 13-24.

TODOROVA, J., MITKOVA, A., BAKALOVA, A., IVANOV, O., DOLAPCHIEV, L., MICHAILOVA, P. 2000. The effect of Cr(NO3)3 on two model species of the family Chironomidae, Diptera – heat shock response and heat shock proteins 70. Biologia, Bratislava, 55/6, 709-716.

WETSEL, R., MCINTOSH, A., ATCHINSON, G. 1978. Evidence of resistance to metal in larvae of the midge *Chironomus tentans* in a metal contaminated lake. Bull. Environ. Contam. Toxicol, 20, 451-455.

WINDER, C., BONIN, T. 1993. The genotoxicity of lead. Mutation Research, 285, 117-124.

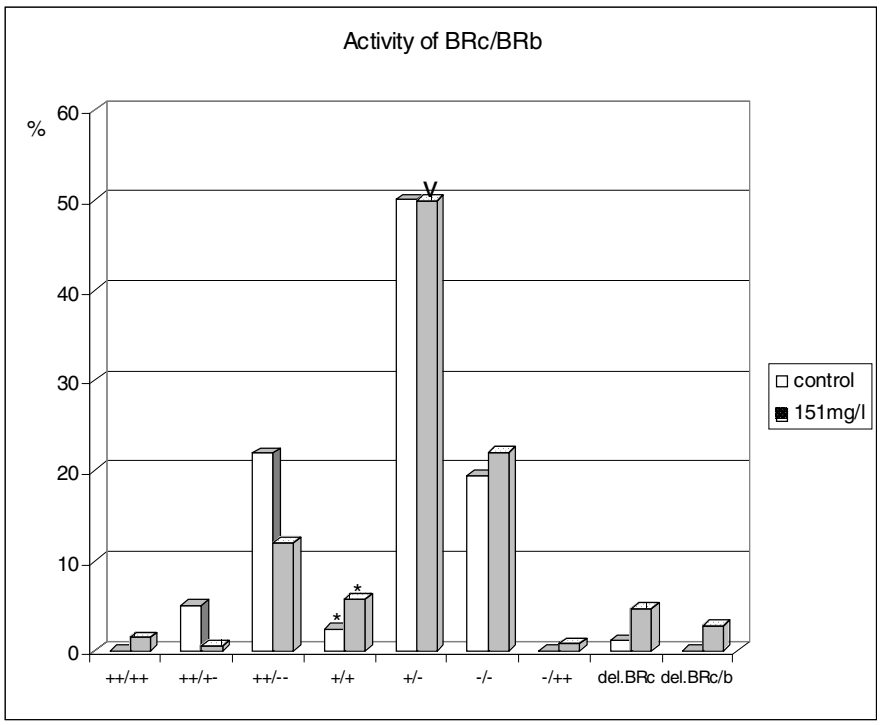

Fig.1a. Levels of activity of BRc/BRb in the *C.piger* polytene chromosomes.

* - Significant difference between control and treated material

V - Significant difference between +/- state of BRc/b and the others in treated material

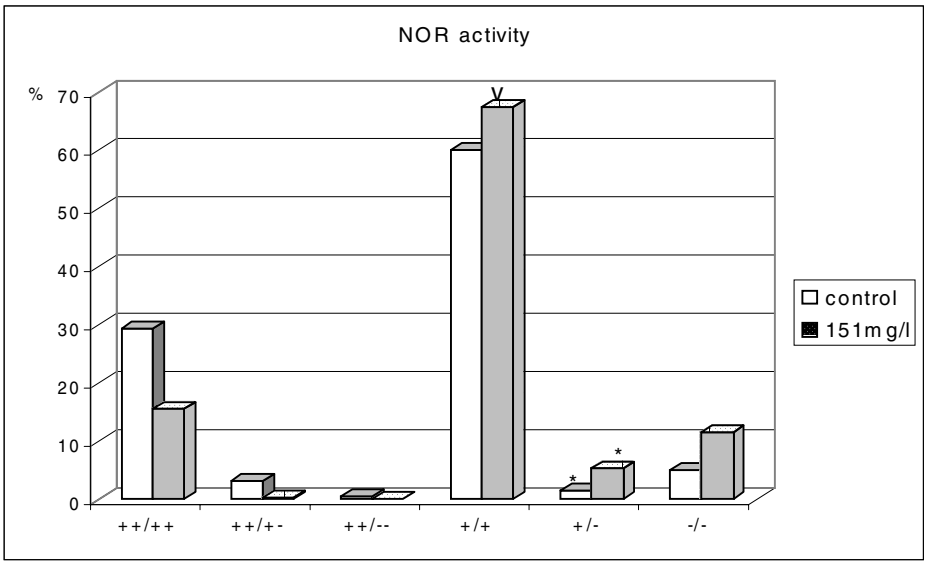

Fig.1b. Different levels of NOR activity
* - Significant difference between control and treated material
V - Significant difference between +/+ state and the others in treated material

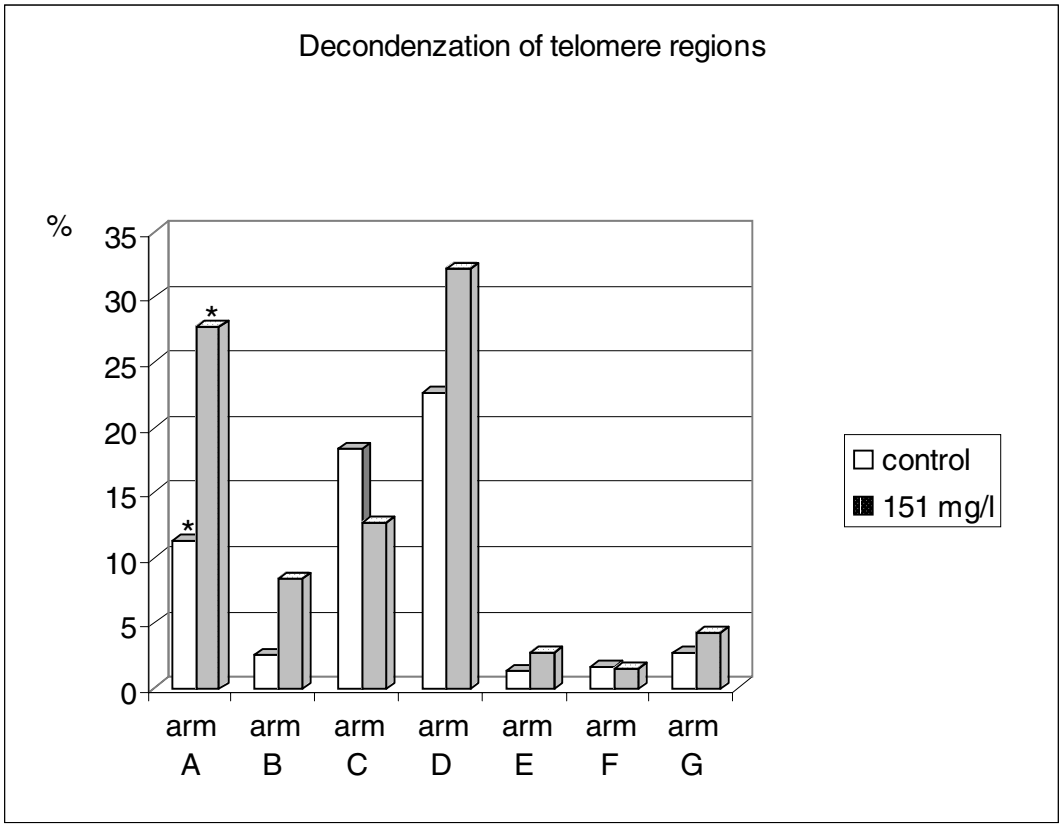

Fig.2. Frequency of decondenzation in telomere regions.
* - Significant difference between control and treated material

Table 1. The number of studied individuals and cells

	Number of individuals	Number of cells
control	27	300
151 mg/l $Pb(NO_3)_2$	33	404