Polytene chromosomes of Chironomidae (Diptera) as a bioassay of trace-metal-induced genome instability

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Chironomids are a ubiquitous group of aquatic insects that are very sensitive to environmental stress. Due to the presence of polytene ('giant') salivary gland chromosomes, it is possible to define the genome response of several Chironomid species to various stress agents. The aim of this study was to assess the genotoxic changes in populations of widely distributed chironomid species from aquatic basins in Bulgaria, Italy, Russia, U.K. and Poland, which were exposed to high concentrations of trace metals. We analyzed the structural and functional alterations of polytene chromosomes of the salivary glands of larvae belonging to three different cytocomplexes of the genus *Chironomus* ("thummi", "lacunarius", "pseudothummi"), and genera *Glyptotendipes* and *Kiefferulus.* Somatic structural chromosome rearrangements (para- and pericentric heterozygous inversions, deletions, deficiencies and amplifications) were used to estimate a Somatic index (S) for each population. The highest S indexes were detected in *Chironomus riparius* populations from locations with high concentrations of trace metals in the sediment. Each species showed specific genome responses to stress agents which we discussed in the light of the specific DNA structures and cytogenetic characteristics of the species. In larvae from polluted sediments two key structures of the salivary gland chromosomes (Balbiani Rings and Nucleolar Organizer) sharply reduced their activity to levels below those observed under non-polluted conditions. It is concluded that polytene chromosomes can be used as tools for evaluating the genotoxicity of the aquatic environment. Structural and functional chromosome alterations provide cost-effective early-warning signals of genotoxic concentrations of environmental pollutants.

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INTRODUCTION

Environmental damage is one of the most critical problems in environmental protection and conservation. Environmental monitoring and assessment of freshwater ecosystem conditions are focused on the analysis of contaminant concentrations and their impact on various biological organisms. For this purpose, Chironomidae (Diptera) are considered a very suitable group. It has been included in all the commonly used biotic indexes (De Pauw et al. 1992) and in the EU Water Framework Directive (Annex V.1.2.6). When used as a bio-monitoring model for eco-toxicological tests, they reveal a strong link between larval morphological deformities and chemical contaminant

concentrations (Warwick 1988). Chironomid larvae (the life history stage that is exposed to aquatic contaminants) have giant (polytene) salivary gland chromosomes with a well-characterized band structure that permits precise cytogenetic analysis and a clear definition of cytogenetic damage. Therefore, the presence of environmental mutagens can be detected in many species by comparing the salivary gland polytene chromosome structure and function of larvae from polluted stations with the standard cytogenetic characteristics of normal larvae (Hägele 1970; Michailova 1989; Kiknadze et al. 1991).

Polytene chromosomes are very sensitive to a variety of physical and chemical stress agents (Diez et al. 1990; Michailova et al. 2009 a, b, c). Michailova et al. (1998) showed that the appearance of somatic aberrations in Chironomid polytene chromosomes is a very promising tool for assessing the genotoxic effects of environmental contaminants at the cellular and population level. Among the Chironomid species, *Chironomus riparius* Meigen, 1804 was chosen by us as a model species because it is widely distributed and has a small number of polytene chromosomes with a clear band structure. In addition, this banding pattern is very well described and mapped (Hägele, 1970; Kiknadze et al. 1991). Other widely distributed species such as *C. piger* Strenzke, 1959, *C. plumosus* (Linne, 1758), *C. bernensis* Wülker, Klötzli, 1973, *C. acidophilus* Keyl, 1960, *Glyptotendipes gripekoveni* Kieffer, 1913, *G. glaucus* (Meigen, 1818) and *Kiefferulus tendipediformis* (Goetghebuer, 1921) also showed genome alterations under different stress agents (Ilkova 2004; Petrova et al. 2004; Michailova et al. 2009 a, b, c; Michailova et al. 2011). These studies revealed that when the concentrations of trace metals in sediments are far higher than those from uncontaminated fossil lake sediments (Forstner, Salomons 1980), numerous somatic chromosome rearrangements can appear. They also showed that two very important structures of the salivary gland chromosomes, the Balbiani Rings (BRs) and the Nucleolar Organizer (NOR), are very sensitive to stress agents (Planello et al. 2007; Michailova et al.2010). BRs correspond to genes that code for the giant proteins which constitute the silk–like scaffold for larval tubes (Wieslander1994). The Nucleolar Organizer is a well defined nuclear compartment in which the transcription of ribosomal DNA (rDNA) leads to synthesis of rRNA and the assembly of ribosomes. The ribosomes play a basic role in protein synthesis.

In the present paper we compared the genome responses to various trace- metal contaminants of larvae from unpolluted sediments to the genome response of larvae from polluted populations of several Chironomid species. To do this, we estimated the frequencies of various types of somatic chromosome rearrangements (para- and pericentric heterozygous inversions, deletions, amplifications and deficiencies) and changes of the functional activity of the Balbiani Rings and Nucleolar Organizers. The number of different types of somatic chromosome rearrangements were used to produce a Somatic index (S) for each species.

MATERIAL AND METHODS

Cytological damages to the polytene chromosomes were analyzed in 4th instar larvae from populations of the following Chironomid species: *C. riparius*, *C. piger, C. plumosus, C. bernensis, C. acidophilus* and *G. gripekoveni, G. glaucus, K. tendipediformis* and a still unnamed *Chironomus* sp. Populations of these species were collected from several geographically isolated water stations in Bulgaria, Italy, Russia, Poland and the U.K, which were characterized by different trace-metal pollutants (Michailova et al. 2005, 2009 a, b, c, 2010; Ilkova 2004; Petrova and Michailova 2002, Sella et al. 2004) (Table 1).

Species of the genus *Chironomus* are grouped in different cytocomplexes. Each cytocomplex is cytogenetically characterized by different chromosome arm combinations. Chromosome arms of species included in cytocomplexes of the genus *Chironomus* are named with the same letter when they have the same or a very similar banding pattern (Keyl, 1962). This indicates homology at cytogenetical level within the same genus. In other genera (e.g. *Glyptotendipes* and *Kiefferulus)* the names of chromosome arms are given following Michailova (1989) and Kiknadze et al (1991) and they do not imply any cytogenetic homology with the chromosome arms of *Chironomus*. For species identification, we used species-specific chromosome or molecular markers, as defined by Hägele (1970), Michailova (1989), Kiknadze et al. (1991) and Michailova et al. (2009 b). All species were studied cytogenetically by applying the conventional aceto–orcein method (Michailova, 1989). The levels of the functional activity of the BRs and NOR were described by means of the Beermann's (1971) qualitative scale.

An index of somatic aberrations (S) was calculated for each population of all the species by dividing the number of different types of somatic aberrations by the number of sampled larvae (Sella et al. 2004; see Table 2). Somatic aberrations (inversions, deletions, deficiencies and amplifications) affected only few cells of a particular individual. Some inversions and deletions observed can be seen in Figure 1 (a, b, c, d). Deficiency is a structural chromosome change that result in the loss of a terminal segment; amplification – repetitious DNA, is expressed by larger than standard bands in the chromosome.

Full details of the preparation and analysis of the chromosomes from the isolated chironomid salivary glands can be found in Michailova (1989).

RESULTS

A wide range of somatic chromosome rearrangements and alterations in the functional activities of polytene chromosomes (Table 2) was found in the populations of the above-listed species from stations with various concentrations of the trace metals listed in Table 1. The structural chromosome aberrations affected all the chromosomes. They were located mainly

Table 1. Trace metal concentrations (µg/g) in the sediments of the stations where Chironomid species were collected.

Species	Stations	Mn	Cu	Cr	Ni	Zn	Pb	Cd	Reference
Fossil sediment	Uncontaminated Lake sediment	406	25	59	51	105	16	0.2	Forstner & Salomons 1980
C. riparius	**Italy** Corio Lat. 45°19'0''N Lon. 7°32'0''E	-	20	35	9.6	-	2.4	-	Sella et al. 2004
	Moncalieri Lat. 45°0'0''N Lon. 7°41'0''E	-	90	56	-	190	12	-	Sella et al. 2004
	Settimo Lat. 45°8'0''N Lon. 7°46'0''E	-	25	240	229	-	13.84		Sella et al. 2004
	Milano Lat. 45°27'50.98''N Lon. 9°11'25.21''E	-	279	114	121	453	76.6	-	Sella et al. 2004
	Santena Lat. 44°57'0''N Lon. 7°47'0''E	-	43	128	200	52	43.2	-	Sella et al. 2004
	Russia St.Petersburg Lat. 59°53'39''N Lon. 30°15'51''E	-	86	32	68	280	88	-	Sella et al. 2004
	Bulgaria Varna Lat. 43°13'0''N Lon. 27°55'0''E	-	41.6	59.5	262	275	19	-	Sella et al. 2004
	Pancharevo Lat. 42.6° Lon. 23.4166667°	-	21	11	6	245	645	-	Sella et al. 2004
	Gabra Lat. 42.583333° Lon. 23.63333°	-	26.4	21.1	21.3	381	270	-	Sella et al. 2004
C. riparius	Maritsa River Lat. 42.083° Lon. 24.817°	1885.4	120.6	45.2	-	-	388.2	16.8	Michailova et al. 2010
	Chaya River Lat. 41.89944° Lon. 24.74389°	435.7	314.3	70.4	-	-	585.9	7.3	Michailova et al. 2010
C. piger	Kokalyane Lat. 42°58'3''N Lan. 23°41'7''E	-	44	111	-	117	451	-	Michailova et al.2009b
C. plumosus	Jana Lat. 42°33'0''N Lon. 23°56'7''E	836	43.5	54.4	57.1	154	95.2	2.5	Ilkova 2004
C. plumosus	**Poland** CzovzsztRes.stat.2 Lat. 49°29'0''N Lon. 20°5'0''E	370	37.5	841.5	38.8	-	26.6	0.7	Michailova et al. 2009c
	CzovzsztRes.stat.3 Lat. 49°29'0''N Lon. 20°5'0''E	120.5	12.9	186.9	16.1	-	12.5	0.56	Michailova et al. 2009c
C. bernensis	**Italy** Santena Lat. 44°57'0''N Lan. 7°47'0''E	-	43	128	200	52	43.2	-	Petrova & Michailova 2002
C. bernensis	**Poland** Dunajec River Lat. 49°29'0''N Lon. 20°5'0''E	288	29.9	40.7	33.9	288	21.1	1.27	Michailova et al. 2009c
C. acidophilus	**UK** Afon Goch River Lat. 53°25'N Lon. 4°25' W	1900	5300	-	-	3800	-	-	Michailova et al. 2009c
Chironomus sp., *Glyptotendipes gripekoveni, Kiefferulus tendipediformis* (cytotype 2)	**Poland** Boleslaw Lat. 50°17'26.61''E Lon. 19°26'36.93''N	-	128.1	-	-	2164.9	528.4	2.4	Michailova et al.2005
G. glaucus	**Bulgaria** Kremikowzi Lat. 42°78'33''N Lon. 23°5'0''E	781	44.2	41.4	45.7	400	21.3	12.6	Ilkova 2004

Lat. – Latitude; Lon.- Longitude

Table 2. Somatic chromosome rearrangements in populations of various Chironomid species.

Species	Station	*N.* of studied individuals	*N.* paracentric inv.	*N.* pericentric inv.	*N.* deletions	*N.* deficiencies and amplifications	Total *N.* aberrations	S index
C. riparius	**Italy** Corio	49	6	-	-	-	6	0.12
C. riparius	Moncalieri	56	1	-	1	2	4	0.07
C. riparius	Settimo	23	13	-	-	-	13	0.57
C.riparius	Milano	86	41	-	-	-	41	0.48
C. riparius	Santena	56	24	5	10	7	36	0.82
C. riparius	**Russia** St. Petersb.	51	2	-	3	4	9	0.09
C. riparius	**Bulgaria** Varna	53	48	3	2	6	59	1.11
C. riaprius	Pancharevo	39	29	3	3	1	36	0.92
C. riparius	Gabra	31	14	2	-	-	16	0.52
C. riparius	Maritsa River	13	8	3	2	-	13	1
C. riparius	Chaya River	14	15	3	3	1	22	1.57
C. piger	**Bulgaria** Kokalyane	27	11	3	3	-	17	0.63
C. plumosus	**Bulgaria** Jana	20	2	-	-	-	2	0.1
C. plumosus	**Poland** Czorsztyn Station 2	13	7	2	-	1	10	0.77
C. plumosus	**Poland** Czorsztyn Station 3	12	5	1	-	-	6	0.50
C. bernensis	**Italy** Santena	14	-	-	-	3	3	0.21
C. bernensis	**Poland** Dunajec River	12	9	-	-	2	11	0.92
C. acidophilus	**U.K.** Afon Goch	35	8	3	1	3	15	0.42
Chironomus sp.	**Poland** Boleslaw	15	5	-	1	1	7	0.47
G. gripekoveni	**Poland** Boleslaw	8	3	-	-	3	6	0.75
K. tendipediformis (cytotype 2)	**Poland** Boleslaw	20	7	-	-	3	10	0.50
G. glaucus	**Bulgaria** Kremokowzi	23	2	-	-	2	4	0.17

N. - number

in the proximal region of the chromosomes. Each somatic rearrangement occurred in a low number of cells per salivary gland and affected a few bands of the chromosomes (Figure 1a, b, c, d).

Structural chromosome aberrations

Genus *Chironomus* Meigen, 1803. The *Chironomus* species that we analyzed belonged to three different cytocomplexes.

1. Cytocomplex "thummi" with chromosome set 2n = 8 and arm combinations AB, CD, EF and G (Keyl 1962). From this cytocomplex, populations of three different species were analyzed: *C. riparius*, *C. piger* and *C. plumosus*. In these species, chromosomes AB and CD are metacentric, while chromosome EF is submetacentric and chromosome G is acrocentric. In species of this cytocomplex, chromosome G carries the Nucleolar Organizer (NOR) and the Balbiani Rings (BRs). The BRs are called BRa, BRb and BRc in *C. piger* and *C. riparius*, and BR_2 and BR_3 in *C. plumosus*. In this species BR_1 is in chromosome arm B.

The genome of *C. riparius* was very sensitive to stress agents in the various polluted stations. Populations from sediments of the five Bulgarian stations, which had the highest concentrations of trace metals, also had the highest values of the Somatic Index (Table 2, see also Figure 1 a). All the analyzed larvae had one or more than one somatic chromosome rearrangement. Somatic rearrangements were also found in the population of *C. piger* from the anthropogenically polluted water station of Kokalyane, (Bulgaria) (Ilkova et al. 2007). The chromosome G of both species was especially sensitive to trace metals and deletions were found which sometimes affected both BRb and BRc, or either BRb or BRc (Figure 1 c, d). Sometimes chromosome G in both species was converted into the so called "pompon" chromosome as a result of these aberrations (4.7% of larvae in *C. riparius* and 3.9 % of larvae in *C. piger*) (Michailova et al. 1998; Michailova et al. 2009 b). Although the populations of *C. riparius* we analyzed were geographically isolated, there were a number of somatic aberrations that had "common" breakpoints. i.e. breakpoints that occurred in the same site in more than one individual either of the same or of different populations (Bovero et al. 2002). Common breakpoints can be considered "weak points" or "hot spots" for breakages. In the *C. riparius* populations 25% of breakpoints were common breakpoints. In the *C. piger* population from Kokalyane (Michailova et al. 2009 b) 22% of the breakpoints were common breakpoints. In both species such "hot spots" occurred more frequently in sections containing blocks of repetitive DNA, whether they were composed of satellite DNA or transposable elements (Bovero et al. 2002).

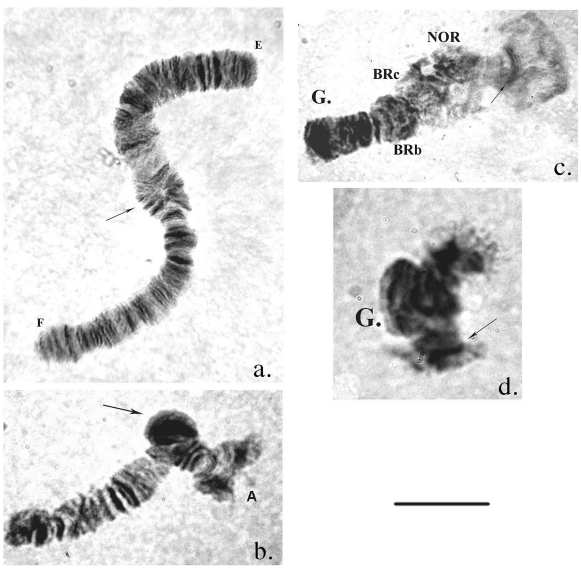

Figure 1. Somatic chromosome rearrangements. a.: Pericentric heterozygous inversion in *C. riparius;* b.: Paracentric heterozygous inversion in *C. acidophilus*; c.: Chromosome G of *C. riparius* - BRc, BRb and NOR with intermediate activity according to the Beermann (1971) scale; d.: Chromosome G of *C. riparius* with deletions of BRb, BRc and some bands in section A. (The arrow in a. and b. indicates the aberrations, while that in c. and d. indicates the centromere region). BR – Balbiani ring and NOR – Nucleolar Organizer. Bar – 100 µm

C. plumosus larvae were collected in the two trace-metal polluted stations in the Polish Czorsztyn reservoir (Michailova et al. 2009 c), where various types of somatic chromosome rearrangements (para- or pericentric inversions and deficiencies) were observed. The Somatic indexes of the larvae from these stations were higher than the S index estimated from the Bulgarian population of Jana (Table 2) (Ilkova 2004). This difference probably results from the Polish stations having sediments with overall higher concentrations of trace metals (especially Cr) than the Bulgarian site (Table 1).

2. Cytocomplex "lacunarius" with chromosome set 2n = 8 and chromosome arm combinations AD, BC, EF and G (Wülker & Klötzli 1973). This cytocomplex includes *C. bernensis*, the only species that was analyzed. Its chromosomes AD and BC are metacentric, chromosome EF is submetacentric and chromosome G is telocentric. Two NORs are located in chromosome AD and EF, respectively, and one BR is in chromosome G. (Petrova & Michailova 2002) In the *C. bernensis* populations from Santena (Italy) and the Dunajec River (Poland), both with sediments polluted by different concentrations of trace metals, a wide range of somatic rearrangements was detected (Michailova et al. 2009 c). At the Polish station, the concentrations of trace metals were higher than those at the Italian station (Table 1) and, accordingly, the Somatic Index at the Polish station was four times higher than that at the Italian station (Table 2).

3. Cytocomplex "pseudothummi" has a chromosome set 2n = 8 and chromosome arm combinations AE, BF, CD and G (Keyl 1962). Two species were analyzed: *C. acidophilus* and *Chironomus* sp. Chromosome AE is submetacentric, chromosomes BF and CD are metacentric and chromosome G is acrocentric. In both species, chromosome G has two BRs and

one NOR. In both species, different somatic rearrangements were observed (Table 2). (Figure 1 b). In *Chironomus* sp. a deletion in chromosome G converted it into a pompon chromosome.

4. Genus *Glyptotendipes* Kieffer, 1918**.** Two species of this genus were found in trace-metal-polluted stations: *G. glaucus* (Ilkova 2004) and *G. gripekoveni* (Michailova et al. 2011) with chromosome set 2n = 8 and chromosome arm combinations AB, CD, EF and G. Chromosomes AB, CD and EF are metacentric; chromosome G is acrocentric. Two BRs and one NOR are located in chromosome G. The population of *G. gripekoveni* inhabited an old mine site near Krakow (Poland). The population of *G. glaucus* inhabited a far less polluted site (Table 1). In both species heterozygous paracentric inversions and deficiencies were found (Table 2) and the values of the Somatic indexes paralleled the very different levels of concentrations of trace metals in the sediments of the two stations.

5. Genus *Kiefferulus* Goetghebuer, 1922. *K. tendipediformis* (cytotype 2) has a chromosome set 2n = 6 with chromosome arm combinations AB, CD, GEF (Michailova et al. 2005). Arm G is fused with chromosome EF (Michailova et al. 2005). BRs and one NOR are located on chromosome G. The second NOR is on chromosome arm A.

A population of this species was found in an old mine site not far from Krakow containing high concentrations of Zn, Pb and Cu (Table 1). Somatic chromosome rearrangements were mainly heterozygous paracentric inversions and deficiencies and they affected all the chromosomes (Table 2).

Functional alterations

Changes in chromosome function were observed along with alterations in the structure of the polytene chromosomes. The important nuclear structures BRs and NOR were very sensitive to stress agents in the environment. The activity of these structures significantly decreased in most of the populations of the species studied (Michailova et al. 1998, 2009 a, 2010; Ilkova 2004, Petrova et al. 2004) to levels lower than those they showed in larvae from unpolluted sediment (Kiknadze 1978). For instance, in *C. riparius* from Pancharevo (Bulgaria) the normal high level of activity of BRc and BRb occurred in less than 10% of the examined cells, while the intermediate or depressed levels of activity were observed in more than 86% of the cells (Petrova et al. 2004). The same tendency towards a depressed level of activity of BRs and NORs occurred in other Chironomid species. For instance, in the *C. acidophilus* population from Afon Gogh UK the standard high level of activity of BRs was observed in only 30.5% of the examined cells, while a depressed level was observed in more than 68.5% of cells (Michailova et al. 2009 a). In some species, species-specific responses were also observed in the functional changes of these structures. For instance, in *C. bernensis*, the changes of NOR's functional activity affected only NOR_2 whose activity, generally very high,fell to full repression. In *K. tendipediformis* (cytotype 2) the functional activity of BR_1 decreased in 97% of the cells while BR_2 remained active (Michailova et al. 2011). In 20% of larvae of the population of *Chironomus* sp and in 11% of larvae of the population of *C. acidophilus,* a new puff occurred near the telomere of chromosome G. In both populations, when such new puffs occurred, BR_2 was not expressed (Michailova et al. 2009 a, 2011). In *C. riparius* a high sensitivity of heterochromatin to stress agents was established (Michailova et al. 1997). A process of decondensation of centromere and telomere heterochromatin was observed in the polytene chromosomes of most of the studied Chironomid species (Petrova et al. 2004; Michailova et al. 2009 c, 2011).

DISCUSSION

The salivary gland chromosomes of all the species studied were very sensitive to trace-metal stress. The resulting genetic damage caused by environmental agents was seen clearly in the salivary glands as functional alterations and structural chromosome abnormalities. As for functional alterations, in all the species studied, the active sites of both BRs and NOR were affected and their normal activity was diminished (Michailova et al. 1998, 2009 a; Ilkova 2004; Petrova et al. 2004). This reaction indicates that both structures are very sensitive and responsive to environmental stressors and can be used to monitor effects of genotoxic agents on the cell metabolism. However, two kinds of functional alterations require further investigation at the molecular level: 1. the appearance of a cytogenetically well identified puff functioning in some larvae of the two species of the same cytocomplex, *C. acidophulus* and *Chironomus* sp., when BR_2 was not expressed; 2. the frequent appearance of centromere heterochromatin in a decondensed state.

A wide range of structural somatic rearrangements (para– and pericentric heterozygous inversions, deletions, deficiencies and amplifications) was found in species of different genera collected from trace- metal polluted sediments (Table 2). In all the species studied the trace metals induced somatic paracentric heterozygous inversions, while somatic pericentric heterozygous inversions, as well as deletions, deficiencies and amplifications were detected in some species only (*C. riparius, C. piger, C. plumosus* and *C. acidophilus*). Moreover, the deletions affecting the chromosome G and in most cases converted it into a pompon in the populations of *C. riparius, C. piger and Chironomus sp.* living in polluted water basins. Such pompons have never been observed in larvae from unpolluted populations of *C. riparius* (e.g. in the populations of Corio and Settimo, Italy). Although the frequencies of pompons are lower than those of somatic inversions, they can be considered as signs of the presence of trace-metal pollution.

When we compared the genome sensitivity of different species, we also found species-specific responses, which can be explained by the differences in DNA organization in each species genome, including differences in the genetic damage-

repair system (Garcia-Sagredo 2008). For instance, the sibling, homosequential species *C. riparius* and *C. piger* belong to the same cytocomplex (thummi). However, their genome structure has many differences in repetitive DNA location. For instance, the copy number of the transposable element NLRCth1 in the phylogenetical younger species *C. riparius* is approximately twice that observed in the older species, *C. piger* (Michailova et al. 2009 c), and the frequency of the minisatellite Alu clusters are twenty times higher in *C. riparius* than in *C. piger* (Ilkova et al. 2007). Since chromosome rearrangements occur mainly at sites which are rich in repetitive DNA elements (King 1993), the frequency of hot spots for chromosome breakages in populations of *C. riparius* is expected to be higher than that in populations of *C. piger*. Differences in the rate of chromosome rearrangements were also found in some *Drosophila* species and were related to different specific amounts of dispersed repetitive DNA (King 1993). Species-specific responses were evident in larvae of *C. bernensis* and *C. riparius*, which occurred sympatrically in the same polluted area (Santena, Italy). The two species belong to different cytocomplexes (*C.riparius,* to "thummi"; *C. bernensis,* to "lacunarius") with different chromosome arm combinations and different genetic backgrounds. This could have influenced their different responses to the same stressor. In *C. riparius* larvae both BRs and NOR appeared to be very sensitive to pollution and often deletions in the G chromosome converted it into a pompon. In contrast, in *C. bernensis* larvae, only NOR_2 in arm E appeared to be sensitive and no changes were observed in the activity of BRs. In *C. bernensis* no pompons were observed, but a "dark knob" was formed in 33% of larvae.

The wide range and high frequency of somatic chromosomal rearrangements observed in populations of different Chironomid species from polluted stations are related to the genotoxic concentrations of polluting agents in the sediments of these stations. When the concentrations of some trace metals in a station were higher than those in another station, cytogenetic damages were s also higher in the first station. This tendency can be clearly seen in populations of *C. riparius*. In the five Bulgarian stations which had the highest concentrations of some trace metals (Table 1), the values of the S indexes were among the highest (Table 2). In contrast, in the populations of the unpolluted or slightly polluted stations of Moncalieri and Corio (Italy) and St. Petersburg (Russia) low concentrations of trace metals had a very weak effect on the chromosomes and the values of the S indexes of these populations were very low. Generally, when the concentrations of the pollutants are not high, different protective mechanisms may mask or repair the action of low concentrations of genotoxic agents (Garcia-Sagredo 2008).

We therefore suggest to that the S index is used as an indicator of metal-derived environmental stress. In addition, this index could be used in routine environmental assessment of genotoxicity and in spatial or temporal comparisons between different populations of the same species, as required by the EU Water Framework Directive.

In conclusion, the cytogenetic approach to environmental monitoring for genotoxicity and the use of the S index allowed us to evaluate the cytogenetic damage with a high degree of resolution. The cytogenetic approach is an economical, quick and valuable tool that can be used with various chironomid species.

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REFERENCES

Beermann W. 1971. Effect of alfa-amanitine on puffing and intranuclear RNA synthesis in *Chironomus* salivary glands. Chromosoma 34: 152–167.

Bovero S, Hankeln T, Michailova P, Schmidt E, 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.

De Pauw N, Ghetti PF, Manzini P, Spaggiari R. 1992. Biological assessment methods for running waters. In: River water quality – Ecological assessment and control, European Commission, Brussels, 217-248.

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

Forstner U, Salomons W. 1980. Trace metal analysis on polluted sediments. I. Assessment of sources and intensities. Environmental Technology Letters 1: 494-505.

Garcia-Sagredo JM. 2008. Fifty years of cytogenetics: a parallel view of the evolution of cytogenetics and genotoxicology. Biochemica and Biophysica Acta 1779: 363-375.

Hägele K. 1970. DNA Replikationmuster der Speicheldrusen Chromosomen von Chironomiden. Chromosoma 31: 91-138.

Hankeln T, Schmidt E. 1990. A new foldback transposable element TFB1 found in histone genes of the midge *Chironomus riparius*. Journal of Molecular Biology 215: 477-482.

Ilkova J. 2004. Chromosome variability of species of subfamily Chironominae (Diptera, Chironomidae) from polluted regions near Sofia. Acta Zooologica Bulgarica 56: 15-30.

Ilkova J, Hankeln T, Schmidt E, Michailova P, Sella G, Zampicinini GP, Petrova N, White K. 2007. Genome instability of *Chironomus riparius* Mg. and *Chironomus piger* Strenzke (Diptera, Chironomidae). Caryologia 60: 299-305.

Keyl H. 1962. Chromosomenevolution bei *Chironomus*. II.

Chromosomenumbauten und phylogenische Beziehungen der Arten. Chromosoma 13: 496-541.

Kiknadze II. 1978. Comparative characteristics of puffing pattern in salivary gland chromosomes of *Chironomus thummi* during larval development and metamorphosis. II. Puffing pattern in chromosomes I, II, III. Tsitologia 20: 514-521.

Kiknadze II, Shilova A, Kekris I, Shobanov N, Zelenzov N, Grebenjuk F, Istomina A, Praslov B. 1991. Karyotype and morphology of larvae in Chironomini. Atlas. Novosibirsk 117 pp.

King M. 1993. Species evolution: role of chromosome changes. Cambridge, Cambridge University Press, UK 358 pp.

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

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

Michailova P, Petrova N, Sella G, Ramella L, Bovero S. 1998. Structural functional rearrangements in chromosome G in *Chironomus riparius* Meigen (Diptera, Chironomidae) collected from a heavy metal polluted area near Turin, Italy. Environmental Pollution 103: 27- 35.

Michailova P, Kownacki A, Warchalowska Sliwa E, Gwiazda E. 2005. Two types of *Kiefferulus tendipediformis* (Goetghebuer, 1921), (Diptera, Chironomidae). Caryologia 58: 62- 69.

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

Michailova P, Ilkova J, Hankeln T, Schmidt E, Selvaggi A, Zampicinini GP, Sella G. 2009 b. Somatic breakpoints, distribution of repetitive DNA and non-LTR retrotransposon insertion sites in the chromosomes of *Chironomus piger* Strenzke (Diptera, Chironomidae). Genetica 135: 137-148.

Michailova P, Szarek Gwiazda E, Kovnacki A. 2009 c. Effect of Contaminants on the Genome of some species of genus *Chironomus* (Chironomidae, Diptera) living in Sediments of Dunajec River and Czorsztyn Reservoir. Water, Air and Soil Pollution 202: 245-258.

Michailova P, Atanasov N, Ilkova J, Chassovnikarova T, Duran M, Karadurmus E. 2010. Genome response of model invertebrates and Vertebrates species to stress agents in the Environment. Biotechnololgy & Biotechnological Equipment 24: 39-396.

Michailova P, Warchalowska Sliwa E, Szarek-Gwiazda E, Kownacki A. 2011. Does biodiversity of macroinvertebrates and genome response of Chironomidae larvae (Diptera) reflect heavy metal pollution in a small pond? Journal of Environment Monitoring and Assessment DOI 10.1007/s10661-011-1942-9.

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

Petrova N, Michailova P, 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: 40-48.

Sella G, Bovero S, Ginepro M, Michailova P, Petrova N, Robotti CA, Zelano V. 2004. Inherited and somatic cytogenetic variability in Palaearctic populations of *Chironomus riparius* Meigen (Diptera, Chironomidae). Genome 47: 332-244.

Warwick WF. 1988. Morphological deformities in Chironomidae (Diptera) larvae as biological indicators of toxic stress. In: Evan, M.S. (Ed.) Toxic Contaminants and Ecosystem Health. A Great Lakes Focus, John Wiley and Sons 80pp.

Wieslander L.1994. The Balbiani ring multigene family: coding sequences and evolution of a tissue-specific function. Proceedings Nucleic Acid Research 48: 275-313.

Wülker W, Klötzli M. 1973. Revision der Gattung *Chironomus* Meig. IV. Arten des lacunarius Komplexes. Archive für Hydrobiologie 72: 474-489.