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Effect of Contaminants on the Genome of Some Species of Genus *Chironomus* (Chironomidae, Diptera) Live in Sediments of Dunajec River and Czorsztyn Reservoir

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Abstract The effect of contaminants on the genomes of *Chironomus plumosus* L. and *Chironomus bernensis* Wülker, Klötzli species was studied in the Dunajec River (station 1) and in the littoral of Czorsztyn Reservoir (stations 2 and 3). According to the index of geoaccumulation, the sediments were polluted by cadmium (Cd) and chromium (Cr). Cd concentrations at stations 1, 2, and 3 were 0.71, 0.56, and 1.27 μg g^{-1}, while Cr concentrations were 841.5, 186.9, and 40.7 μg g^{-1}, respectively. The pH of sediments ranged from 6.7 to 7.1. Genome instability of *C. bernensis* and *C. plumosus* L. was evaluated at the stations and was determined by structural and functional chromosome alterations. Living in sediment containing higher concentrations of Cr and elevated concentrations of Cd (station 1), *C. bernensis* was found to possess a high spectrum of somatic chromosome rearrangements with a somatic index of 0.92. *C. plumosus* collected from stations 2 and 3 was found to have somatic indices of 0.77 and 0.5, respectively. Both species mobilized their genome by changes in their functional activity: the appearance of novel puffs and the participation of chromosomes in an ectopic pairing (more common in *C. bernensis* than in *C. plumosus*). The observed chromosome alterations were interpreted as a response of the genome to stressful conditions in the environment. The study indicates that the salivary gland chromosomes of the observed species provide a sensitive system for the easy tracking of genome changes induced by stressful conditions.

Keywords Chironomidae · Chromosome alterations · Cr · Cd · Reservoir · River

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1 Introduction

This study examined the effect of heavy metal concentrations on selected animals in an aquatic ecosystem. Aquatic insects are very sensitive bio-indicators of heavy metals contamination because they are exposed to the contaminants during their embryogenesis, larval development, and pupation. Their larvae inhabit the sediment where the accumulation of heavy metals occurs. One of such insect groups, very sensitive to environmental stress and widely distributed in aquatic ecosystems, is a species of genus *Chironomus*. We have shown that the genome of these species is much more responsive than the phenotype to environmental stress and thus

provides an ideal early warning system for the presence of different pollutants (Michailova et al. 1996, 2000). Our studies under controlled laboratory conditions and at polluted sites in different natural water basins have shown that environmentally relevant concentrations of trace metals result in cytogenetic damage in a number of species from the genus *Chironomus* (Michailova et al. 2001a, b, 2003a, b, 2006, 2007). The aim of this work was to present the effect of contaminants (mainly heavy metals) on the genome of *Chironomus bernensis* Wülker, Klötzli and *Chironomus plumosus* L. (Chironomidae, Diptera) living in sediments of Dunajec River and Czorsztyn Reservoir (southern Poland).

2 Study Areas

The Dunajec River is a left tributary of the Vistula River; it has its source in the Tatra Mountains at an altitude of 1,650 m above sea level (ASL) and joins the Vistula River at 170 m ASL. Its total length is 247 km and its watershed area measures 6,798 km^2. At the 173.3 km point of the Dunajec River, a dam was constructed creating the Czorsztyn Reservoir (Fig. 1). Above the reservoir, the Dunajec River is highly polluted by sewage from the leather tanneries. The tanneries' wastewater is flushed directly into the Dunajec River (and its tributaries), or into local sewage treatment plants. In many cases the plants are not equipped to effectively treat tannery waste (Szalińska 2002). The highest Cr load is released into the Dunajec River during autumn and winter, when leather production is at its peak (Szalińska 2002; Szalinska et al. 2003).

The Czorsztyn Reservoir was built in 1997 and is one of the highest situated dam reservoirs in Poland (standard dam level—529 ASL). It is 11 km in length, has an area of about 1,051 ha, a mean depth of 19 m (maximum about 46 m), and a capacity of 181.2 mln m^3. The average water exchange occurs 3.3 times a year (Mazurkiewicz-Boroń 2002). Its watershed area measures 1,147 km^2.

The water quality of the Dunajec River reflects the natural regional background and the management of the watershed. In the Dunajec River above and below the reservoir and in the Białka River (a tributary of the reservoir), bicarbonates predominate (68% of total

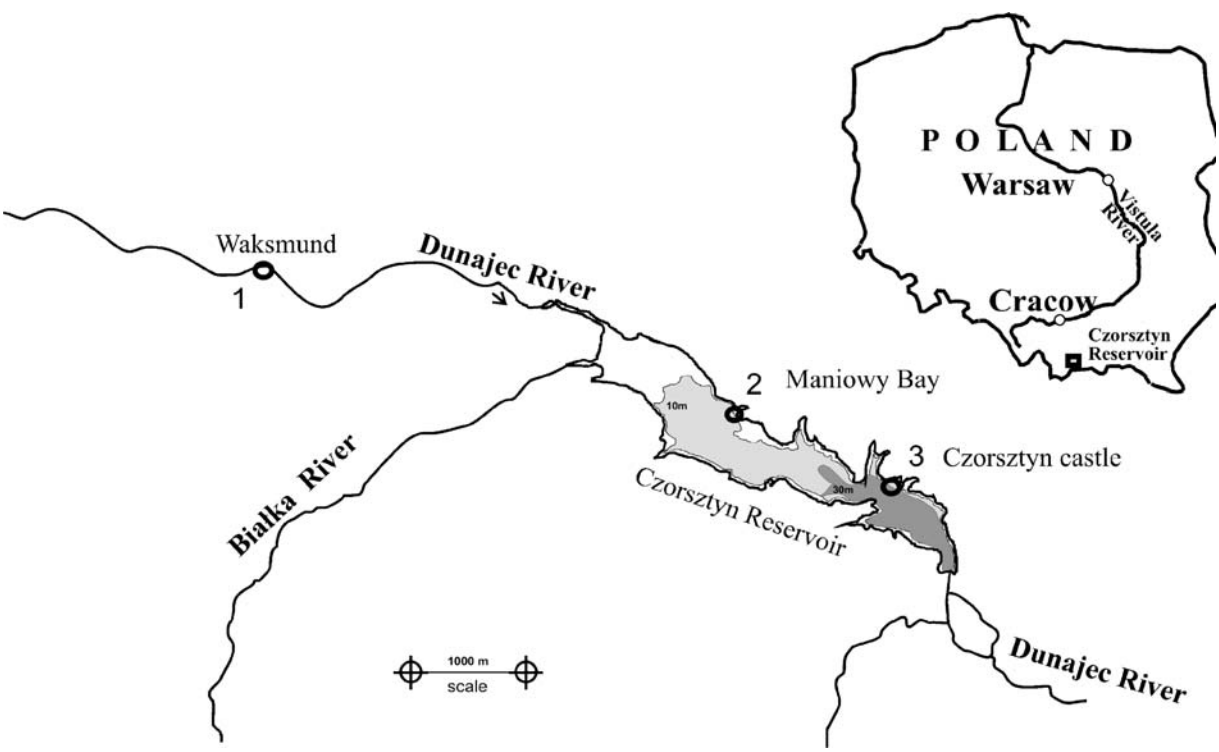

Fig. 1 Location of sampling sites in the Dunajec River and Czorsztyn Reservoir

anions) over sulfates (15–23%) and chlorides (8–11%). The sulfate and chloride amounts decrease in the Czorsztyn Reservoir compared to those found in the upper section of the Dunajec River due to dilution effected by the Białka River. Among cations, Ca (66–69% of the total cation) and Mg (22–25%) predominate over Na (5–9%) and K (1–2%; Szalińska and Dominik 2006). The pH of the Dunajec River and Czorsztyn Reservoir is alkaline (Mazurkiewicz-Boroń 2002; Szalińska and Dominik 2006). In the water of the Dunajec River and Czorsztyn Reservoir, heavy metals (except Cr) were detected in low concentrations, in the range of a few micrograms per liter (Szalińska and Dominik 2006). Chromium was found in the Dunajec River in extremely high concentrations (Szalińska 2002; Pawlikowski et al. 2006; Dominik et al. 2007). In its most polluted section, close to tannery discharges in the village of Waksmund, in the period of the highest discharge of wastewater from the tannery, Cr (III) exceeded 85.2 μg dm^{-3}, while Cr (VI) was much lower (0.015–4 μg dm^{-3}; Szalińska 2002). Chromium concentrations in Dunajec River sediments in Waksmund were also very high (1,600 μg g^{-1}; Pawlikowski et al. 2006).

3 Materials and Methods

3.1 Sample Collection

Larvae of genus *Chironomus* and sediment samples were collected from three stations in July 2007 (Fig. 1). Station 1 was situated in the Dunajec River above the reservoir, near the mouth of the canal carrying wastewater from a small tannery in Waksmund. Station 2 was located at the Czorsztyn Reservoir in shallow Maniowy bay. In its vicinity, a sewage plant and small tannery are located. Station 3 was situated at the Czorsztyn Reservoir near the popular tourist destination of Czorsztyn Castle. The list of species, the number of examined individuals, and their cells are given in Table 1.

3.2 Analytical Methods

The pH, organic matter, and total heavy metal contents (Cd, Pb, Cu, Cr, Ni, Mn, and Fe) were determined in sediment samples. The pH of wet sediments was measured with an Elmetron pH meter (CX-742). Organic matter content was determined by the ignition of a sediment sample at 550°C for 2 h and expressed as loss of ignition (LOI).

Table 1 Number of analyzed larvae and cells of *C. bernensis* and *C. plumosus* collected from Dunajec River (station 1) and Czorsztyn Reservoir (stations 2 and 3) on 3 July 2007

Species	Localities	Number of larvae analyzed	Number of cells analyzed
Chironomus bernensis	Station 1	12	267
Chironomus plumosus	Station 2	13	352
Chironomus plumosus	Station 3	12	302

Sediment samples used to determine the total heavy metal concentrations were dried at 105°C and sieved with a 0.200-mm sieve. Approximately 0.5 g of dry sediment (three subsamples from each station) were digested with 15 ml conc. HNO_3 in tubes on the heated block of the Tecator Digestion System 12, in conjunction with an Autostep 2000 controller set to 120°C for 2 h. Heavy metal concentrations in sediment were determined by flame or graphite furnace atomic absorption spectrophotometer using a Varian Spektr AA-20. Analytical accuracy for sediment samples was assessed using Standards Reference Material (NCS DC 73308). Comparisons of measured and certified values of analytical standards concentrations are given in Table 2.

3.3 Cytogenetic Methods

The fourth instar larvae were preserved in alcohol and glacial acetic acid (3:1). Each larva was used for cytogenetical and morphological analysis. Isolated salivary glands were squashed for chromosome preparations, which were prepared according to Michailova (1989). All preparations with clear polytene chromosome structure were prepared as permanent slides. The preparations of external morphology of larvae and polytene chromosomes are maintained in the Institute of Zoology, Bulgarian Academy of Sciences. Applying a cytotaxonomical approach, we identified the species by cytogenetical markers (Michailova 1989; Kikinadze et al. 1991). In station 1, *C. bernensis* Wülker, Klötzli was identified by mapping of chromosomes according to Petrova and

Table 2 Comparison of measured and certified values of analytical standards NCS DC 73308 concentrations

	Cd (μg g^{-1})	Pb (μg g^{-1})	Cu (μg g^{-1})	Cr (μg g^{-1})	Ni (μg g^{-1})	Mn (μg g^{-1})
Measured values	1.23±0.03	28.9±0.15	20.6±1.02	130.5±5.1	28.9±0.6	985±5.6
Certified values	1.12±0.08	27±2	22.6±1.3	136±10	30±2	1010±29

Michailova (2002). In two other locations (stations 2 and 3), *C. plumosus* L. was found. Mapping of the polytene chromosomes of this species was done according to Butler et al. (1999). In analyzing the genome response, we considered both the inherited and somatic aberrations. An inversion is accepted as inherited when it affects all cells of both salivary glands in the same individual and as somatic when nuclei with and without that inversion are detected in both salivary glands (Sella et al. 2004).

For both species, the presence of inherited homozygous and heterozygous combinations of the banding sequences was shown, for instance, as plu A1.1 or plu A1.2 for *C. plumosus* and ber A1.1 or ber F2.2 for *C. bernensis*. When the aberrations were somatic in appearance, they were indicated in the following way: the arm of the chromosome, the type of aberration, and the section where it was present.

3.4 Statistics

To estimate the contamination status of the sediment, the index of geoaccumulation (I_{geo}) was calculated according to Müller (1981):

$$I_{geo} = \log_2(C_n/1.5\ B_n)$$

where C_n is the mean concentration of an element in bottom sediment and B_n is the geochemical background of the element in the shale (Turiekien and Wedepohl 1961). Müller (1981) distinguished seven categories of sediment contamination from unpolluted (category 0; $I_{geo}<0$) to extremely polluted (category 6; $I_{geo}>5$).

For each locality frequencies of genotypic combinations for both species were recorded as percentages. The degree of somatic structural chromosome aberrations in each location was estimated by dividing the number of somatic aberrations found in that location by the number of studied individuals. This ratio is referred as the somatic (S) index according to Sella et al. (2004).

4 Results

4.1 Sediment

Chemical characteristics of the sediments of three studied stations are presented in Table 3, while the values of geoaccumulation index (I_{geo}; according to Müller 1981) for the elements in Table 4. In general, the sediment was characterized by low amounts of organic matter (expressed as LOI). Higher amounts of organic matter were found at station 1 near the input of tannery sewage as compared to those in stations 2 and 3 situated at the Czorsztyn Reservoir. In turn, the

Table 3 Heavy metal concentrations in the bottom sediments (fraction <0.200 mm) of the Dunajec River (station 1) and Czorsztyn Reservoir (stations 2 and 3) in July 2007 and in reference sediment according to Förstner and Salomons (1980)

	SDF reference sediment	SFF reference sediment	Station 1	Station 2	Station 3
pH			7.1	6.7	6.9
LOI (%)			10.6	3.1	6.1
Cd (μg g^{-1})	0.2	0.3	0.71±0.06	0.56±0.07	1.27±0.05
Pb (μg g^{-1})	16	30	26.6±0.85	12.5±0.14	21.1±0.18
Cu (μg g^{-1})	25	51	37.5±0.4	12.9±0.9	29.9±0.5
Cr (μg g^{-1})	59	47	841.5±7.7	186.9±0.1	40.7±0.6
Ni (μg g^{-1})	51	46	38.8±1.3	16.1±0.3	33.9±1.2
Mn (μg g^{-1})	406	960	369.8±9.1	120.5±4.4	287.8±2.5
Fe (mg g^{-1})	18.2	32.35	14.0±0.6	5.5±0.6	13.6±0.4

SDF sediment fossil lake, *SFF* sediment fossil river

Table 4 Index of geoaccumulation (I_{geo}) of heavy metals in the bottom sediments of the Dunajec River (station 1) and Czorsztyn Reservoir (stations 2 and 3) in July 2007

	Station 1	Station 2	Station 3
Cd (μg g^{-1})	0.7[a]	0.3[a]	1.5[d]
Pb (μg g^{-1})	−0.2[b]	−1.3[b]	−0.5[b]
Cu (μg g^{-1})	−0.9[b]	−2.4[b]	−1.2[b]
Cr (μg g^{-1})	2.6[c]	0.5[a]	−1.7[b]
Ni (μg g^{-1})	−1.4[b]	−2.7[b]	−1.6[b]
Mn (μg g^{-1})	−1.8[b]	−3.4[b]	−2.2[b]
Fe (mg g^{-1})	−2.3[b]	−3.7[b]	−2.4[b]

[a] I class—slightly polluted

[b] 0 class—unpolluted

[c] III class—strongly polluted

[d] II class—moderately polluted

sediment at station 3 contained twice the amount of organic matter than sediment at station 2. At all of the stations, the pH of sediments was about neutral (Table 3).

Higher concentrations of Pb, Cu, Ni, Mn, and Fe were two to three times greater than the lowest concentration of the aforementioned metals among the stations studied (Table 3). The highest concentrations of Pb, Cu, Ni, Mn, and Fe in the sediment (but according to I_{geo} still considered an unpolluted concentration; Table 4) were found at station 1 near the inflow of sewage from the tannery, while the lowest were found at station 2. The values of I_{geo} indicated that the sediment was slightly polluted by Cd at stations 1 and 2 and moderately polluted at station 3 (Table 4). Cr pollution of sediments ranged from unpolluted (station 3), through slightly polluted (station 2), to strongly polluted (station 1). The concentration of Cr in sediments at station 1 near the inflow of sewage from the tannery was about 21 times higher as compared to that found at station 3.

4.2 Cytogenetic Characteristics

4.2.1 C. bernensis Wülker, Klötzli

The diploid chromosome set is $2n=8$, with chromosome arm combinations: AD, BC, EF, and G (Fig. 2a–d). It has two nucleolar organizers (NOR): in arm A and arm E, as has been described by Wülker and Klötzli (1973). One Balbiani ring (BR) is localized in arm G. Chromosomes AD and BC are metacentric, chromosome EF is submetacentric, and chromosome G is telocentric. The centromere region of all chromosomes is easily seen and is marked by dark heterochromatin bands. The banding patterns in arms A, B, C, D, E, and G in the studied population (Fig. 2a–d) do not differ from the standard (berA11, berB11, berC11, berD11, berE11, berG11). These banding patterns and the location of the BRs and NOR (which are species specific) coincided with those found in Swiss and Italian populations (Wülker and Klötzli 1973; Petrova and Michailova 2002). The studied specimens have a homozygous inversion in arm F (berF22)—an aberration which has been found in other Polish populations (Michailova et al. 2002).

4.2.2 C. plumosus L.

The chromosome set is $2n=8$, with chromosome arm combinations AB CD EF G. Chromosomes AB and CD are metacentric, EF is submetacentric, and chromosome G is acrocentric (Fig. 3a–d). It has two Balbiani rings (BR) in chromosome G. The Balbiani ring in arm B, which is characteristic of the species, is not expressed in the studied population. In addition, one Nucleolar organizer (NOR) is located in chromosome G, as has been described by Butler et al. (1999).

The centromere regions are expressed either by a thin dark band or by a block of large heterochromatin bands. The banding sequences of the chromosomes are pluA11, pluB11, pluC11, pluD11, pluE11, pluF11, and pluG11 and do not differ from the standard (Butler et al. 1999).

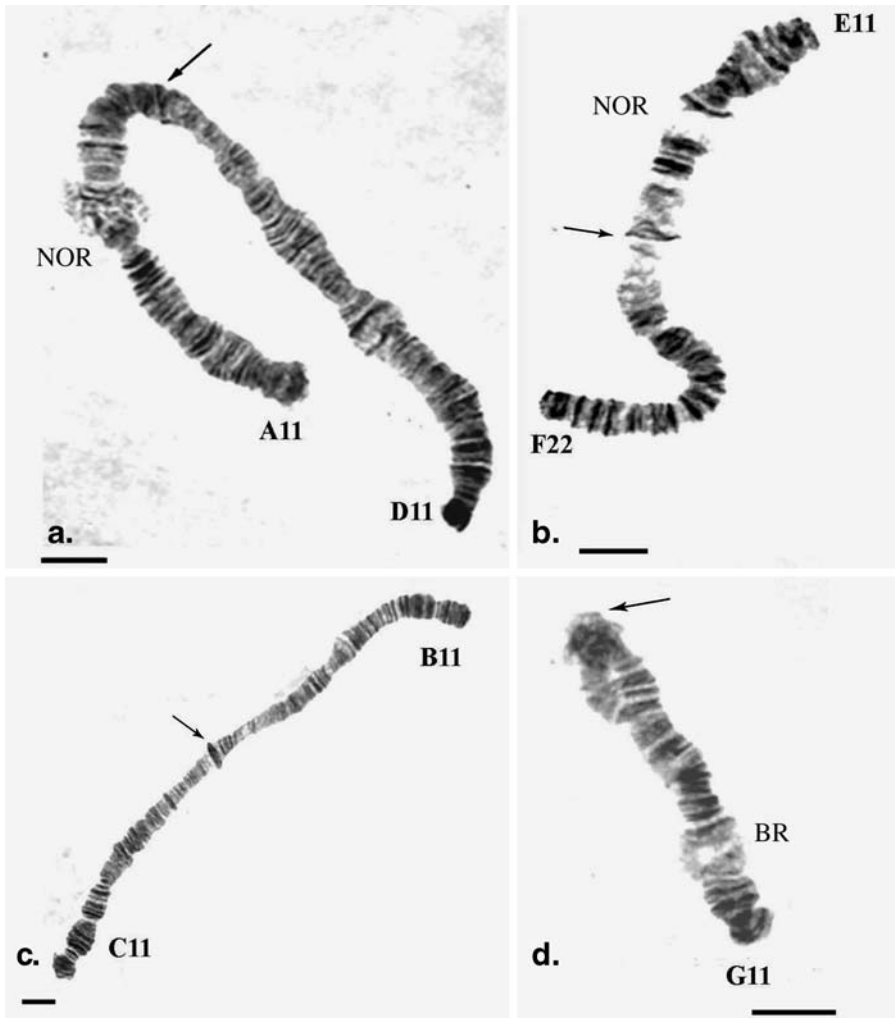

Fig. 2 Standard polytene chromosomes of *C. bernensis* Wülker, Klötzli. **a** Chromosome A11D11; **b** chromosome E11F22; **c** chromosome C11B11; **d** chromosome G11. *NOR* nucleolar organizer, *BR* Balbiani ring. *Arrow*—the location of the centromere regions; *bar* 10 μm

4.3 Structural Chromosome Rearrangements

4.3.1 C. bernensis

Only one inherited inversion—arm F (F1.2; 0.08%; Fig. 4c)—was observed. This inversion has been detected in Swiss and Italian populations (Wülker and Klötzli 1973; Petrova and Michailova 2002). In total, 11 new different somatic rearrangements were detected: two deficiencies, eight paracentric heterozygous inversions, and one chromatid break (Figs. 4a, b, d, e; 5a, b; and 6c).

The different types of heterozygous inversions and their frequencies are given in Table 5. The value of the S index is 0.92. In 3.75% of the studied cells, the centromere heterochromatin of chromosome G occurred as a "dark knob". In some cells, it appeared in the heterozygous state (33.33%; Table 5; Fig. 6b).

4.3.2 C. plumosus

In two localities, the same type of inherited inversion was found: pluD1/D2. In station 2, it had a lower frequency (23.08%) than in station 3 (58.33%). In addition, a genomic polymorphism characterized by an additional B chromosome was found in both localities. It had a higher frequency in station 3 (16.7%) and lower in station 2 (7.7%; Fig. 7). In station 2, one individual had a chromosome fragment always associated with chromosome AB.

In total, ten somatic chromosome rearrangements were detected in *C. plumosus* in station 2 and only six in station 3. Their frequency and localization in the

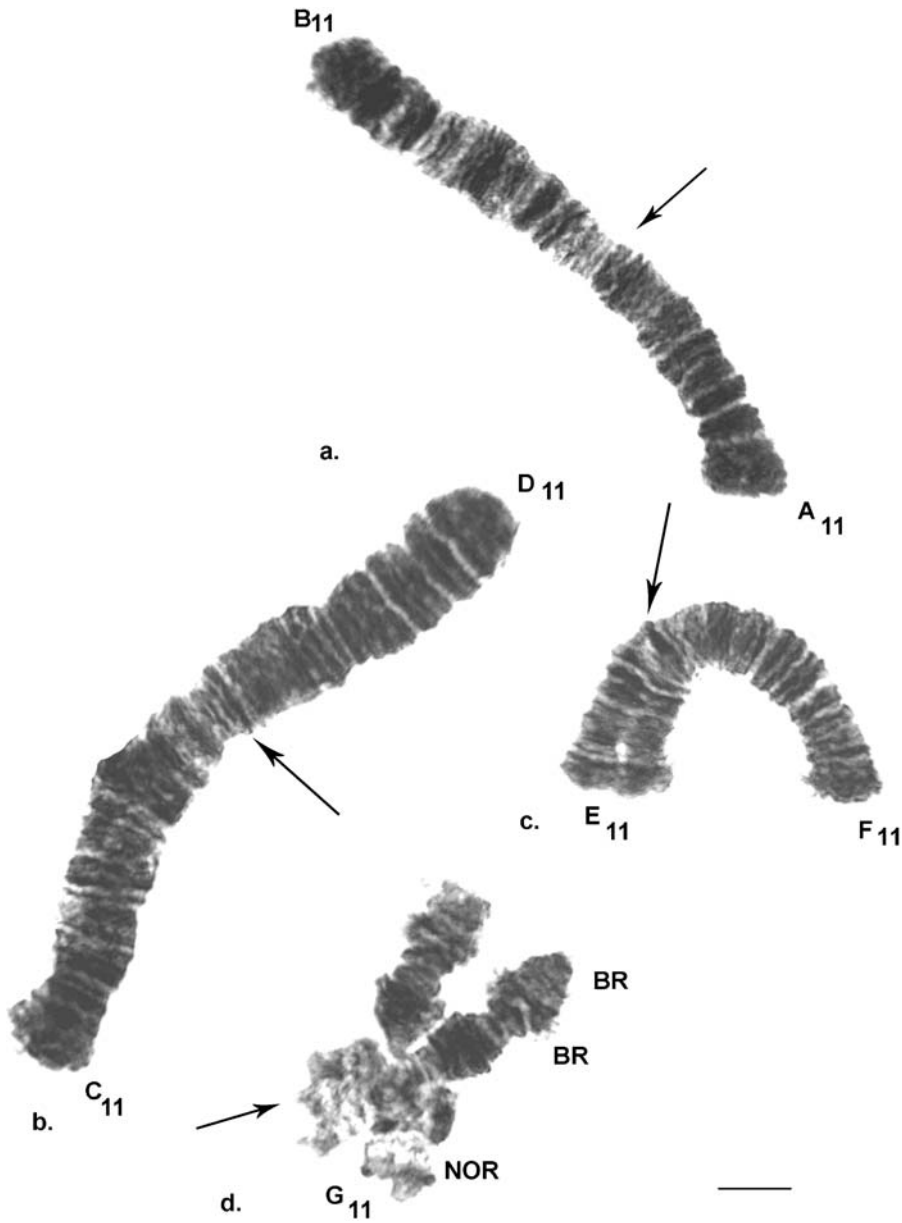

Fig. 3 Standard polytene chromosomes of *C. plumosus* L. **a** Chromosome A11B11; **b** chromosome C11D11; **c** chromosome E11F11; **d** chromosome G11. *NOR* nucleolar organizer, *BR* Balbiani ring. *Arrow*—the localization of the centromere regions; *bar* 10 μm

chromosome arms is shown in Table 6. It is important to underline that they are simple inversions, affecting a few cells only. They are new for *C. plumosus* and were not previously found in *C. plumosus* at other localities studied (Butler et al. 1999; Krastanov and Michailova 2008). In both localities, they affected the same section of the chromosomes (Fig. 8a–d; Table 6). The S index in *C. plumosus* at station 2 is 0.77, while at station 3 is 0.5.

The centromere regions of *C. plumosus* of both studied localities were expressed in three different states: thin bands, large heterochromatin bands, and banding in the heterozygous state (Fig. 9a–c). In both localities, the highest frequency of the heterozygous state of centromere regions was found in chromosome EF: in station 2—17.89%; in station 3—9.60%, followed by CD: station 2—9.65% and station 3—8.28%; and chromosome AB: station 2—4.26% and station 3—1.65%. In both localities, there are individuals where the centromere region of chromosome G was in heterozygous state in individual cells.

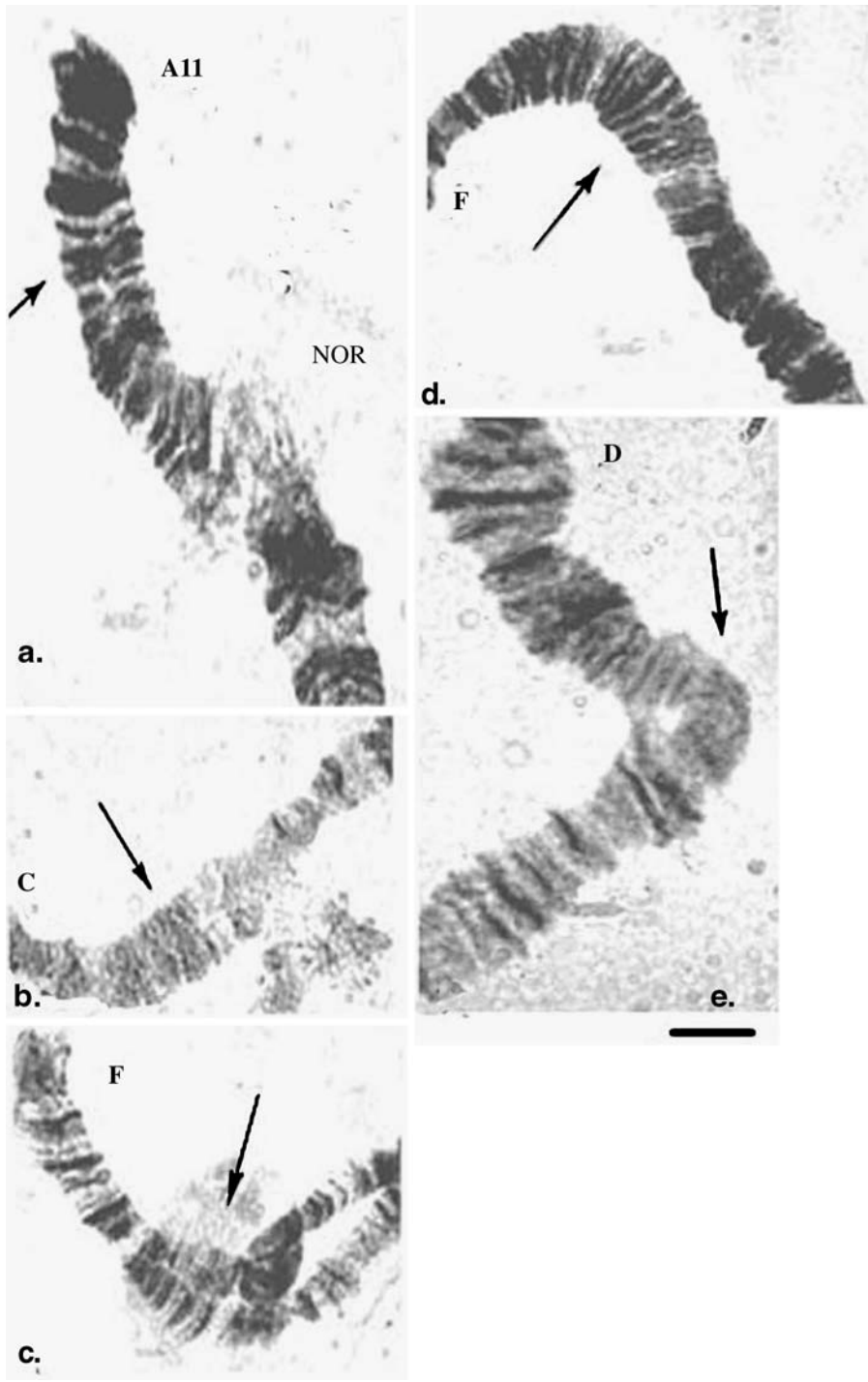

Fig. 4 Aberrations in *C. bernensis* Wülker, Klötzli. **a** Somatic heterozygous inversion in arm A, sections 7–8; **b** somatic heterozygous inversion in arm C, sections 11–12; **c** inherited heterozygous inversion in arm F, sections 11–5d; **d** somatic heterozygous inversion in arm F, sections 12–13; **e** somatic heterozygous inversion in arm D, sections 23–24. *NOR* nucleolar organizer. *Arrow*—the location of the inversion; *bar* 10 μm

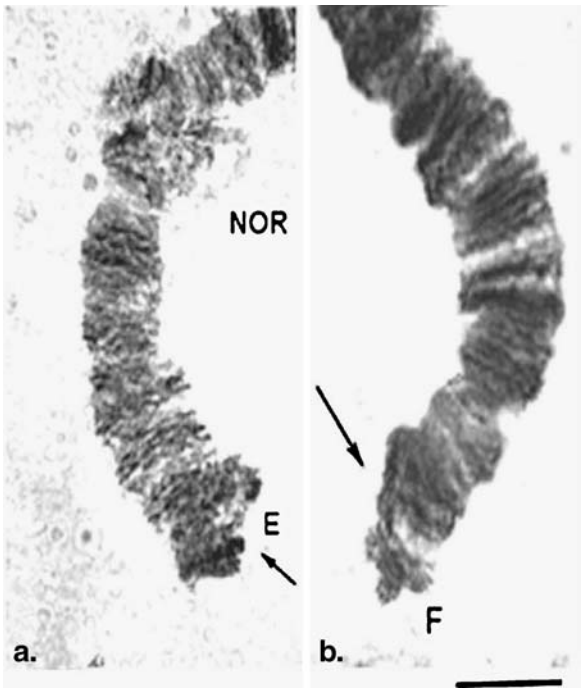

Fig. 5 Deficiencies in chromosome EF of *C. bernensis* Wülker, Klötzli. **a** Deficiency in arm E; **b** deficiency in arm F. *NOR* nucleolar organizer. *Arrow*—the localization of the deficiency; *bar* 10 μm

4.4 Functional Alterations in the Chromosomes

4.4.1 C. bernensis

Some novel puffs were found in chromosome arm B in 4.87% of the cells and in arm G in 1.87% of the cells (Fig. 6a). Very often, the chromosome arms participated in ectopic pairing: arm G—12.35%, arm D—11.61%; arm A—9.36%; arm B—8.99%; arm F—8.24%, arm E—7.49%, arm C—5.24%. Only telomeres took part in ectopic pairing, appearing mainly as threads. The following combinations of telomeres were observed:

G + E + F: B + A + F: A + F; D + G; B + E; D + E
G + B: C + A + F: E + B + A: G + A + E + B:
G + E: E + D: G + E + D + B.

4.4.2 C. plumosus

In individual cells at both localities, a disturbance of the conjugation of the homologs in chromosome arms C and E was found. On the other hand, both homologs of chromosome G were conjugated at the telomeres: at station 2—21.21% and at station 3—3.64% (Fig. 9d).

5 Discussion

In general, Dunajec River (station 1) and Czorsztyn Reservoir (station 2 and 3) sediments were characterized by low amounts of organic matter (express as LOI) and a nearly neutral pH. According to the geoaccumulation index (Müller 1981), sediments were not polluted by Pb, Cu, Ni, Mn, and Fe and polluted to different degrees by Cd (slightly at stations 1 and 2; moderately at station 3) and Cr (strongly at station 1; slightly at station 2; unpolluted at station 3). The concentration of Cr was 18 times higher at station 1 and three times higher at station 2 while that of Cd was two to three times higher at stations 1 and 2 and six times higher at station 3 as compared with concentrations in the reference sediments (Förstner and Salomons 1980). The highest concentrations of Cr in the Dunajec River sediments points to tannery sewage as the main source of pollution. Pawlikowski et al. (2006) also showed very high Cr concentrations (700–1,600 μg g^{-1}) in

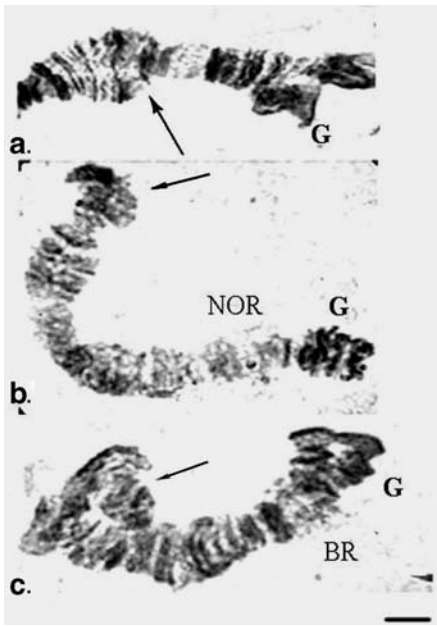

Fig. 6 Chromosome G of *C. bernensis* Wülker, Klötzli. **a** A puff; **b** "dark knob" in heterozygous state; **c** heterozygous inversion in section 3. *BR* Balbiani ring. *Arrow*—the location of the alteration; *bar* 10 μm

Table 5 Somatic aberrations in *C. bernensis* collected from the Dunajec River (station 1)

Chromosome arm	Aberration and its location	Number of individual with aberration	Percent	Number of cells with aberration	Percent
A	Inv.11–12a	2	16.7	2	0.749
A	Inv.7–8	1	8.3	1	0.37
C	Chr.break 14–15	1	8.3	1	0.37
C	Inv.11–12	1	8.3	1	0.37
D	Inv.2	2	16.7	3	1.12
D	Inv.23–24	2	16.7	3	1.12
E	Inv.7–8	1	8.3	1	8.3
F	Inv.12–13	2	16.7	2	0.75
E	Deficiency 1	1	8.3	1	0.37
F	Deficiency 1	1	8.3	1	0.37
G	Het.inv.3	3	25	5	1.87

polluted sediments (fraction<2,000 μm) of the Dunajec River. Such high Cr concentrations are typical for water environments affected by tannery effluent (Pawlikowski et al. 2006; Rodrigues and Formoso 2006; Giusti and Taylor 2007).

To estimate the potential ecological impact of sediment contaminants, the obtained contents of trace elements were compared to the probable effect level (PEL) of the Canadian Environmental Quality Guidelines (CCME 1997). The concentrations of trace elements in the sediment which can cause the probable effect level were established in micrograms per gram of sediment as follows: Cd 3.53, Pb 91.3, Cu 197, Ni 36, and Cr 90. Therefore, concentrations of Cd, Pb, Cu, and Ni in Dunajec River and Czorsztyn Reservoir sediments were below the PEL. The concentration of Cr was about nine times higher at station 1 and about 2 times higher at station 2 than the PEL; therefore, these sediments can be considered to be contaminated and potentially causing toxic ecological effects.

This study showed a high level of chromosome alterations in populations of *C. plumosus* and *C. bernensis* inhabiting polluted aquatic areas in southern Poland. The genome of both species is very sensitive to the influence of environmental pollution. The structural alterations were somatic and inherited. Inherited alterations have been established in other Palaearctic populations (Butler et al. 1999; Petrova et al. 2007; Krastanov and Michailova 2008) and expressed by the chromosome polymorphism of the species, having different selective value, depending on

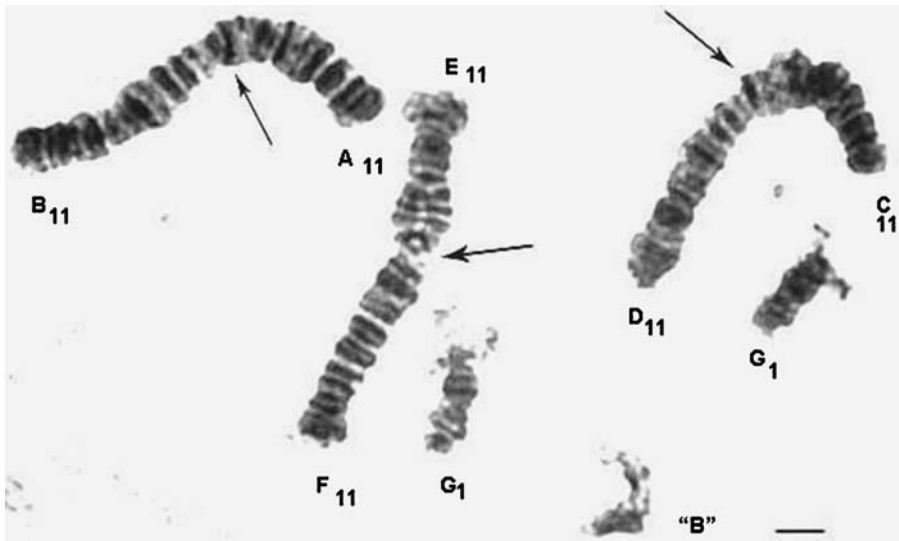

Fig. 7 Polytene chromosome of *C. plumosus* L. and an additional "B" chromosome. *Arrow*—the location of the centromere region; *bar* 10 μm

Table 6 Somatic aberrations in *C. plumosus* from the Czorsztyn Reservoir (stations 2 and 3)

Arm	Localities								
	Aberration and its location	Station 1				Station 2			
		Number of individuals with aberrations	%	Number of cells with aberrations	%	Number of individual with aberration	%	Number of cells with aberration	%
A	Inv.5–6	5	32.46	8	2.56	2	16.67	4	1.32
A	Inv.15–15	2	15.38	2	0.56	–	–	–	–
B	Inv.4–6	2	15.38	3	0.85	1	8.33	2	0.66
C	Inv.17–18	1	7.69	1	0.28	–	–	–	–
E	Inv.7–8	1	7.69	4	1.14	2	16.67	4	1.32
F	Inv.8	4	30.77	4	1.34	2	16.67	2	0.66
F	Inv.12–13	1	7.67	1	0.28	1	8.33	2	0.66
AB	Pericentr.inv	1	7.69	1	0.28	–	–	–	–
EF	Pericentr.inv	2	15.38	2	0.57	1	5.33	1	0.33
E	Deficiency	1	7.69	1	0.28	–	–	–	–

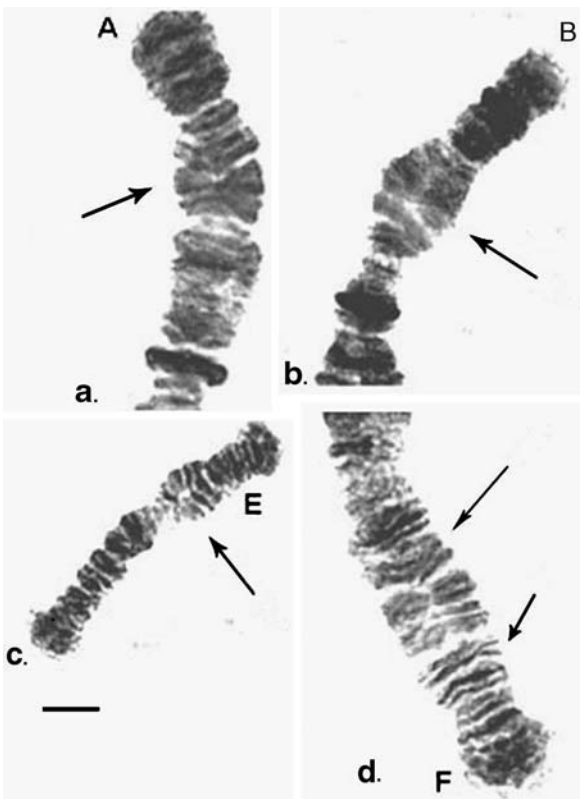

Fig. 8 Somatic chromosome aberrations in *C. plumosus* L. **a** Heterozygous inversion in arm A, sections 5–6; **b** heterozygous inversion in arm B, sections 4–6; **c** heterozygous inversion in arm E, sections 7–8; **d** heterozygous inversion in arm F sections 8 and 12–13. *Arrow*—the location of the alteration; *bar* 10 μm

the environmental components of the studies localities. However, our study established for the first time a high spectrum of somatic chromosome rearrangements in both species collected in water basins heavily polluted by Cr. Lagadic and Caquet (1998) stress that the somatic alterations can be used to assess the genotoxic effect of environmental agents. It is important to stress that in relatively unpolluted basins, *C. plumosus* and *C. bernensis* did not posses any somatic alterations (Wülker and Klötzli 1973; Butler et al. 1999; Krastanov and Michailova 2008). In *C. bernensis*, collected in Northern Italy at stations polluted with trace metals, somatic structural chromosome alterations, different from those observed by us, were also detected (Petrova and Michailova 2002). In additional studies, Sella et al. (2004) showed that the frequency of different somatic alterations in *C. riparius* is directly proportional to the level of heavy metal pollution. Our results confirm this idea and show that the somatic index of *C. plumosus* is higher in more polluted stations than those with less pollution. Somatic aberrations were also observed by us in the second species *C. bernensis*, collected from station 1, where sediment contained high concentrations of heavy metal. In *C. plumosus* from both polluted stations, in addition to the somatic aberrations, a small very condensed B chromosome was found, which has also been considered as pollution marker (Petrova et al. 2007).

The possibility that the somatic alterations in the studied species might be a result of the synergistic

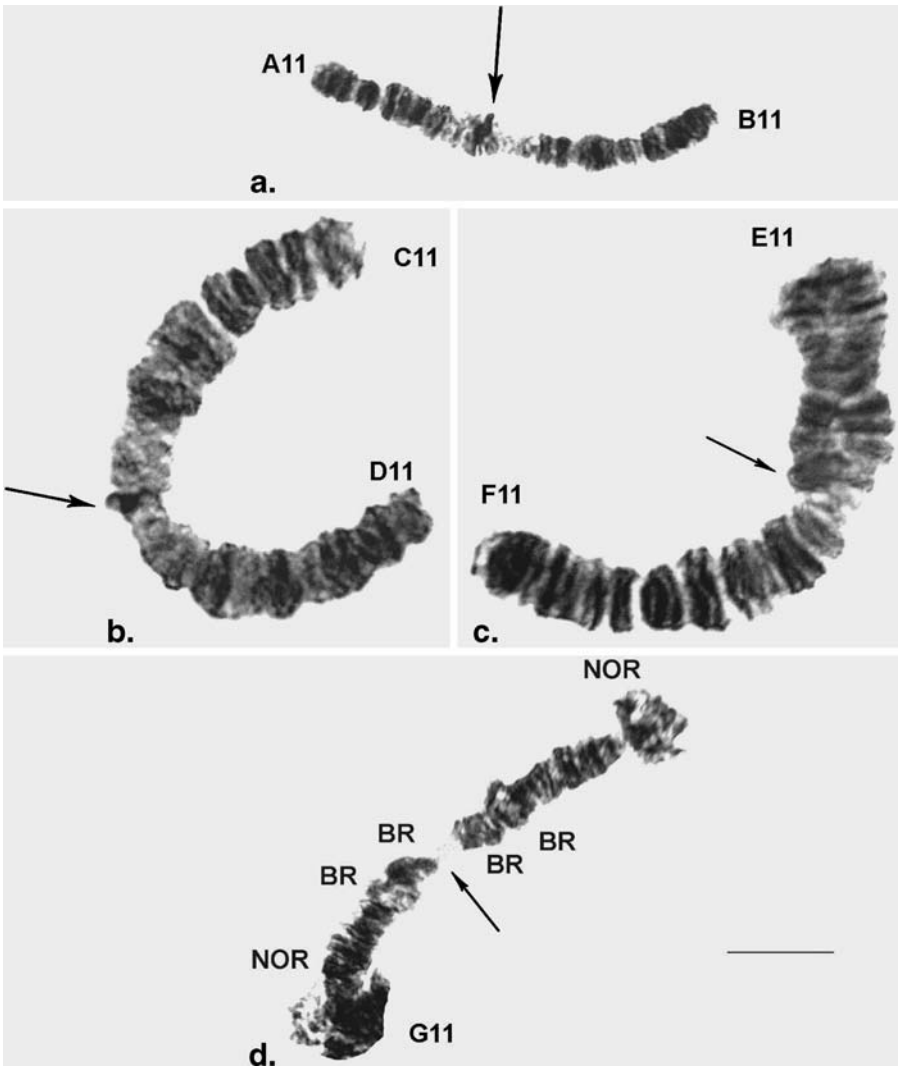

Fig. 9 Polytene chromosomes of *C. plumosus* L. **a** Chromosome AB—centromere region in heterozygous state; **b** chromosome CD—centromere region in heterozygous state; **c** chromosome EF—centromere region in heterozygous state; **d** ectopic pairing between homologous of chromosome G. *Symbols* as in Fig. 8

interaction among the metals cannot be excluded. However, to confirm that heavy metals have a genotoxic effect, it is necessary to perform laboratory experiments using different concentrations of the metals.

In addition to the above mentioned somatic structural chromosome rearrangements, some changes in functional activity are worth additional discussion. Those changes included pairing of the telomeres of *C. bernensis* polytene chromosomes and forming occasionally a ring of chromosomes. *C. plumosus* also displayed ectopic pairings between the homologs of chromosome G. Such pairing of chromosomes was recorded in *Chironomus balatonicus* collected in Chernobyl following the nuclear accident (Michailova and Petrova 1991) and in the river Po (Italy) polluted with trace metals (Michailova et al. 1996). The results support the idea of mobilization of the genome and might indicate the role of mobile elements in this process. We hypothesize that the pairing of the chromosomes through their telomeres promotes the transfer of mobile elements from one chromosome to another and thus influences the activity of the whole genome. A similar phenomenon was described by Ivashchenko et al. (1991) in *Drosophila* where contacts favored the transfer of mobile elements from one chromosome to another and thus enhanced the functional activity of the whole genome.

An interesting cytogenetic phenomenon was observed in *C. bernensis*. In some cells, the centromere regions of chromosome G appeared as a “dark knob”, which also occurred in the heterozygous state. The

same phenomenon has been observed in *C. bernensis* from northern Italy (Petrova and Michailova 2002). Such a "dark knob" has also been found in *Chironomus nuditarsis* (Fischer and Tichy 1980) in Switzerland. The authors considered the specimens with this "dark knob" as a mutant, found in the natural population, where G at its distal part lacks the Balbiani ring and presents a large compact chromatin mass. It is quite possible that in both species, an additional DNA replication occurred. Keyl (1965) suggested that the localized DNA increase can result from spontaneous or induced breakage of the chromatid and the subsequent DNA replication. Michailova et al. (2001b) found a significant amplification in some sections of *C. riparius* genome treated with different Cr concentrations. However, to determine the cause of the "dark knob" in *C. bernensis*, it is necessary to do molecular studies and describe the molecular mechanism of the observed phenomenon.

In conclusion, we found that the salivary gland chromosomes of the studied species represent a very sensitive system for easily tracking genomic changes induced by stressful environments. The observed somatic structural and functional chromosome alterations in the studied species support Lagadic and Caquets' (1998) idea that these alterations can be used as indicators of stressful agents in the environment. Therefore, it is possible to employ a chromosome assay for in situ monitoring of contaminants in aquatic ecosystems. However, the field studies do not give a complete answer as to which metals play a major role in genotoxicity. It is well known that the interaction among the metals may have a synergistic effect on genotoxicity.

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