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Biodiversity of Chironomidae (Diptera) and genome response to trace metals in the environment

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Abstract: The effects of pollution on the biodiversity and genome response of Chironomidae larvae (Diptera, Insect) were evaluated in the trace metal contaminated water environments. No change on the Chironomid species diversity was found. The higher concentrations of trace metals (Cd, Pb, Cr, and Zn) affect the genome of 5 cytogenetically studied Chironomid species: *Chironomus bernensis*, *C. plumosus, C.* sp.1. *Kiefferulus tendipediformis* (cytotype 2), *Glyptotendipes cauliginellus* (syn. *Glyptotendipes gripekoveni*). Genome instability of Chironomid larvae was manifested by two ways: 1. Fixed chromosome rearrangements; homozygous inversions and tandem fusions created new gene linkage groups and show an intensive microevolutionary process of species. 2. Somatic structural (inversions, deficiencies, deletions, breaks); and functional alterations (decreasing the activity of the key structures: Balbiani rings (BRs) and Nucleolar Organizer (NOR)) in salivary gland chromosomes of cytogenetically studied Chironomidae species. Detecting somatic rearrangements in salivary gland chromosomes of these widely distributed aquatic insects is potentially one of the best validated bioassay and can be used as a cost effective early warning signals of environmental damage in freshwater basins.

Keywords: trace metals, biodiversity, somatic chromosome alterations

INTRODUCTION

Chironomidae (non-biting midges) are a ubiquitous group of aquatic insects that are suitable biological indicators of environmental contamination.This group is included in the biotic indices commonly used throughout Europe [1]. There is a strong link between the chironomid phenotype – specifically larval morphological deformities – and chemical contaminants. Additionally, some authors [2, 3] have shown that the salivary gland chromosomes of chironomids are much more sensitive than the phenotype to environmental stress agents. Such cytogenetic changes can be well defined due to large (polytene) salivary gland chromosomes [4] present in chironomid larvae which are exposed to contaminants.

Having in mind the advantages of Chironomidae, we made a hypothesis that trace metal contamination of the sediment increase the genome instability of different Chironomidae species and have some effect on Chironomidae biodiversity in different Poland stations.

MATERIAL AND METHODS

The investigations were carried out in four the southern Poland areas: (1) a pond in Bolesław, (2) Dunajec River (Waksmund) and (3, 4) Czorsztyn Reservoir on the Dunajec River − two stations.

(1) The study was conducted in the small pond (max. depth 1 m) situated on a recent mine spoil in Bolesław (E 50°17’26.61”, N 19°26’36.93”) (Olkusz Region with Zn-Pb ore deposits). In this area, mining started in the 12th century and has continued to this day. Mine spoils are heavily contaminated with heavy metals [5];

(2) The Dunajec River in the village Waksmund (E 20°5’53.6”, N 49°28’51”) is highly polluted by sewage from the leather tanneries. Water and sediment in some parts of the river are heavily contaminated with Cr [6, 7];

(3, 4). In the Czorsztyn Reservoir were located two stations. Station 3 in Maniowy Bay (E 20°15’35”, N 49°27’3”) contaminated mainly with Cr originated from a small sewage treatment plant collecting sewage from local leather tanneries. In the station 4 (E 20°18’42”, N 49°26’9”), situated in a bay near the Czorsztyn castle, the concentration of trace metals was lower than that found in the reference sediment (except Cd ions) (Table 1).

Table 1. Trace metal concentrations (μg g^{-1}) in the sediments studied in the Polish stations and somatic index of the cytogenetically studied Chironomid species

Locations	Cd	Pb	Cu	Zn	Cr	Ni	Cytogenetically studied species	Somatic index
Investigated area								
Pond in Bolesław (Station 1)	7.3	1370	106	1990	26.2	28.6	*Chironomus sp. I*, *Glyptotendipes cauliginellus*, *Kiefferulus tendipediformis* (cytotype 2)	0.47 0.75 0.50
Dunajec River, Waksmund (Station 2)	0,7	26.6	37.5		841.5	38.8	*C. bernensis*	0.92
Czorsztyn Res. (Station 3)	0.56	12.5	12.9	105	186.9	16.1	*C. plumosus*	0.77
Czorsztyn Res. (Station 4)	1.27	21.2	29.9	115	40.7	33.9	*C. plumosus*	0.50
References data of heavy metals concentration according to Förstner & Salomons 1980								
Unpolluted river sediment	0.3	30.0	51.0	115	47	46		-
Unpolluted lake sediment	0.2	16.0	25.0	105	59	51		-
The values of probable effect level (PEL) according to Smith et al. 1996								
Probable effect level	3.53	91.3	197	315	90	36		

Cytogenetic damages (structural and functional rearrangements) to the polytene chromosomes were analyzed in 4th instars larvae from five Chironomid species (Table 2). Preparations of the polytene chromosomes were obtained from squashes of salivary gland cells stained with aceto-orcein [4]. The number of individuals and cells of the Chironomidae species for cytogenetical analysis is presented in Table 2. The degree of somatic variability of each species was estimated by dividing the number of different somatic aberrations observed in that species by the number of sampled larvae [3]. This ratio was considered by Sella et al. [3] as the somatic (S) index.

To assess the biodiversity of Chironomidae, samples were collected with hand-net at stations 1 and 2 and Eckman grab at stations 3 and 4. These materials were determined to species or genus level. Sediment samples (<0.2 mm) were extracted with 65% HNO_3 using microwave Speed Wave (Berghof). Heavy metals (Cd, Pb, Cu, Zn, Cr, and Ni) were established by flame atomic absorption spectroscopy (Varian Spektra AA-20). To assess the accuracy of the analytical method the Sediment Reference Materials (NCS DC 73308, river sediment) was used.

Table 2. Localities and number of studied individuals (Ind.) and cells of different Chironomidae species

Species	Pond in Bolesław (station 1)		Dunajec River (station 2)		Czorsztyn Res. (station 3)		Czorsztyn Res. (station 4)	
	Ind.	Cells	Ind.	Cells	Ind.	Cells	Ind.	Cells
C. bernensis	-	-	12	267	-	-	-	-
C. plumosus	-	-	-	-	13	352	12	302
Chironomus sp. I	15	370	-	-	-	-	-	-
K. tendipediformis (cytotype 2)	20	382	-	-	-	-	-	-
G. cauliginellus (syn. *G. gripekoveni*)	8	256	-	-	-	-	-	-

RESULTS AND DISCUSSION

The sediments studied at the stations were characterized by neutral and slightly alkaline pH (6.7-7.6). The pond in Bolesław was strongly contaminated by Cd, Pb, and Zn, while in the Dunajec River (Waksmund) and the Czorsztyn Reservoir at station 3 by Cr (Table 1). The concentrations of these heavy metals in the studied sediments were considerable higher comparing to the unpolluted water bodies (Table 1) [8]. They also exceeded the "probable effect level" (PEL) [9], which represent the concentration above which adverse effect to organisms is expected to occur frequently (Table 1). The detected high concentrations of Cd, Pb, and Zn in the sediment of the pond in Bolesław were associated with the geochemical background and the neighbouring recent Zn–Pb mine spoil [5], while Cr in the sediments of the Dunajec River in Waksmund and Czorsztyn Reservoir (station 3) with the sewage from the leather tanneries.

Table 3. Diversity of Chironomidae communities in the pond in Bolesław (BP, station 1), the Dunajec River in Waksmund (DR, station 2) and the Czorsztyn Reservoir (CR, stations 3 and 4 are given together) in Southern Poland

Taxa	BP	DR	CR
TANYPODINAE			
Procladius (*Holotanypus*) sp.(l.)		X	
Macropelopia sp.		X	
Ablabesmyia (Ablabesmyia) longistyla Fittkau 1962 (p)	X		
Zavrelimyia sp. (l.)	X		

Thienemannimyia − complex (l.)		X	
Clinotanypus nervosus (Meigen 1818) (p.)	X		
Tanypodinae (juv.)		X	
PRODIAMESINAE			
Prodiamesa olivacea (Meigen 1818) (l.)		X	
ORTHOCLADIINAE			
Corynoneura sp. (l.)	X		
Eukiefferiella sp. (l.)		X	
Paracladius conversus (Walker 1856)		X	
Cricotopus (Isocladius) sylvestris group (l.)	X		
Psectrocladius psilopterus group. (p.)	X		
Orthocladiinae (juv.)		X	
CHIRONOMINAE			
Cryptotendipes sp.(l.)		X	
Dicrotendipes sp. (p.)	X		
Chironomus (Chironomus) luridus Strenzke 1959 (♂. p. l.)	X		
Chironomus sp. I (p.)	X		
Chironomus (Chironomus) bernensis Kloetzli 1973 (gen)		X	X
Chironomus (Chironomus) plumosus (Linnaeus 1758) (gen)		X	X
Chironomus spp. (l.)	X	X	X
Kiefferulus (Kiefferulus) tendipediformis (Goetghebuer 1921) (♂.p.l)	X		
Microtendipes pedellus group		X	
Synendotendipes impar (Walker 1856) (syn. *Endochironomus impar* (Walker 1856))	X		
Glyptotendipes (Glyptotendipes) cauliginellus (Kieffer 1913) (p.) (syn. *Glyptotendipes gripekoveni* Kieffer, 1913)	X		
Stictochironomus sp.(l.)		X	
Polypedilum (*Polypedilum*) spp.(l.)	X	X	
Polypodilum (*Tripodura*) sp. (l.)		X	
Paratendipes sp.(l.)		X	
Chironomini (juv)		X	
Micropsectra atrofasciata (Kieffer 1911) (p)		X	
Micropsectra sp. (l.)		X	
Cladotanytarsus sp. (l.)	X		
Paratanytarsus laccophilus (Edwards 1929) (p)	X		
Paratanytarsus bituberculatus (Edwards 1929) (p)	X		
Paratanytarsus spp. (l.)	X		
Tanytarsus brundini Lindenberg 1963 (p. l.)		X	
Tanytarsus pallidicorris (Walker 1856) (p)		X	
Tanytarsus usmaensis - group. (p.)	X		
Tanytarsus sp. (Pe 4) (according to Lancton 1991) (p.)	X		
Tanytarsus sp. (?Pe 14) (according to Lancton 1991) (p.)	X		
Tanytarsus spp. (l.)		X	
Tanytarsini (juv.)		X	

l. – larvae, p – pupae, ♂ − imago of male, gen – determined on cytogenetical studies.

We did not detect the impact of trace metals on the Chironomids diversity in the studied basins. A total of 35 taxa of Chironomidae were identified: they belong to 26 genera, 19 species in the pond Bolesław, 16 species in the Dunajec River and 2 species in the Czorsztyn Reservoir (Table 3). The diversity of the Chironomidae larvae was typical for a small pond, river with mudded bottom and reservoir. However, very sensitive to trace metals is the genome of the studied Chironomid species. A high spectrum of somatic chromosome rearrangments, as inversions (Figure 1), deficiencies, deletions, breaks, was detected in the salivary gland chromosomes of the studied species [10]. They affected small regions of the polytene chromosomes and few cells of the individual only. It is important to underline that in unpolluted basins the studied species did not possess any somatic aberrations [4]. Lagadic and Caquet [11] proposed somatic rearrangements to be used as biomarkers of stress agents in the environment. On the basis of these chromosome rearrangements the somatic index of every species was calculated (Table 1). We proposed it to be used as an additional biomarker for assessment the pollutants in the environment. Together with this type of aberrations in an old mine station (Bolesław), polluted since 12 century [5] an intensive microevolution process was observed. It is realized by fixed chromosome rearrangements affected all individuals and cells. This process has been detected in two species collected from Bolesław: *Chironomus* sp. and *Kiefferulus tendipediformis*. In Bulgaria and Hungary (unpolluted stations) the species indentified by external morphology as *K. tendipediformis* has 2n = 8 with chromosome arm combinations AB CD EF and G [10]. The same species collected from a highly contaminated area of Bolesław has 2n = 6 with chromosome arm combinations AB CD GEF. The chromosome set is reduced due to fixed tandem aberration by which chromosome G is fused with chromosome EF. In the Bolesław pond a still undescribed new species *Chironomus* sp. has been found. This species belongs to the cytocomplex "pseudotummi"[12] with 2n = 8 and chromosome arm combinations: AE BF CD G. It distinguished from the other species of this cytocomplex by fixed homozygous inversion in arm A and specific band sequences of arms B, C, D and G. The permanent chromosome rearrangements (tandem fusion and homozygous inversion) created new gene linkage groups and show the running an intensive microevolutionary process in the highly contaminated old mine site.

Together with structure chromosome aberrations some functional alterations were established. The important key cells structures Balbiani rings (BRs) and Nucleolar Organizer (NOR) decreased their functional activity. It was known that BRs are very important for normal development of the larvae as they are sites of intensive description of genes encoding for silk proteins [13]. Chironomids used silk proteins for constructions larval tubes where the larvae are developed. In *K. tendipediformis* BR_1 often appeared in collapse or low activity (97.12%). The suppression of the normal high activity of NOR was detected also. This structure is responsible for the synthesis of ribosomal RNA – important for normal cell functions. For instance, in *Chironomus* sp. together with high activity (more than 55%) intermediate activity was found as well (more than 44%).

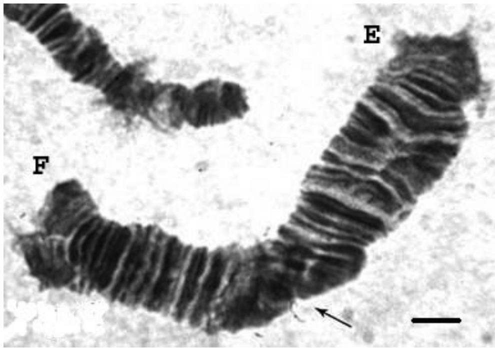

Figure 1. Somatic heterozygous inversion in *Glyptotendipes cauliginellus* (syn. *G. gripekoveni*).
Chromosome EF – a polytene chromosome with somatic heterozygous inversion, indicated by arrow. Bar − 100 µm.

Species-specific response was observed in the functional activity of the studied species. The species of genus *Chironomus* belong to different cytocomplexes [12, 14]: *Chironomus* sp.to "pseudothummi"; *C. plumosus to* "thummi", *C. bernensis* to "lacunarius" with different chromosome arm combinations and different gene linkage groups. This may have influenced their species-specific responses. When BR_2 in chromosome G of *Chironomus* sp. was not expressed, a puff near the Nucleolar Organizer (NOR) was observed. A "dark knob" (33.3%) in heterozygous state was observed at the telomere of the chromosome G in *C. bernensis* collected from the Dunajec River. The centromere regions of the chromosome AB, CD EF in *C. plumosus* from both studied localities occurred in heterozygous state (chromosome EF – 27.49%; chromosome CD – 17.93%; chromosome AB − 5.91%).

So, the results obtained indicate that the genome instability: functional alterations, somatic cytogenetic damage (heterozygous inversions, deletions, deficiencies) and somatic index are particularly suitable as biomarkers: they are cost effective, fast and wide valuable markers in different Chironomidae species.

The impact of trace metals on Chironomids diversity was not established.

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